The role of 3D surface texture measurement of natural human enamel for assessment of erosive tooth wear

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The role of 3D surface texture measurement of natural human enamel for assessment of erosive tooth wear

Thesis submitted for degree of
Doctor of Philosophy

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Abstract

This thesis investigated the role of 3D surface roughness measurement for the quantification of erosive tooth wear. The aim of this thesis was to develop a method for quantifying 3D surface roughness changes occurring in natural unpolished enamel and polished enamel during erosion.

Firstly, an *in vitro* erosion model was developed for quantifying mechanical and optical surface changes of natural unpolished enamel during dietary erosion through a series of pilot studies. Following this a series of validation studies were conducted to identify measurement error (measurement noise, accuracy, precision and software errors) when quantifying surface texture of complex biological structures using optical profilometry. The measurement protocol was validated for measuring 3D roughness over different locations across natural unpolished enamel and polished enamel samples by comparing the results of imaging five central areas (each 0.004 mm^2) over the apex of the curvature of the sample, in comparison to measuring 20 peripheral areas (each 0.004 mm^2) in order to reliably measure surface roughness. The optimised surface texture measurement protocol was then used to quantify surface roughness changes of natural and polished enamel from *in vitro* three cycle erosion regimes (15, 30 and 45 minutes) using a commercially available orange juice drink. Finally, an *in situ* investigation into the effects of the 15, 30 and 45 minutes of erosion times was conducted with acid immersion occurring both *ex vivo* and *in vivo*.

The initial work suggested significant reduction in Sa roughness of natural unpolished enamel following 45 minutes of erosion in orange juice from median (IQR) 0.62 (0.27) µm to 0.38 (0.06) µm ($P<0.01$). Subsequent validation studies revealed no significant differences between the median (IQR) Sa roughness of the central 1.45 (2.58) µm and peripheral areas 1.32 (4.86) µm before erosion for natural unpolished enamel, and whilst the median (IQR) roughness significantly decreased to 0.38 (0.35) µm and 0.34 (0.49) µm respectively ($p<0.0001$) there were no significant differences between measuring the centre or peripheral areas. For polished enamel, there were no significant differences

across the sample with 0.04 (0.17) µm for central and 0.05 (0.15) µm for the peripheral areas before erosion. Whilst Sa roughness significantly increased after erosion to 0.27 (0.08) µm ($p<0.0001$) there were no significant differences between measuring the central or peripheral areas. When investigating three erosion times *in vitro* there were only significant changes in natural unpolished enamel after 45 minutes of erosion: median (IQR) Sa roughness decreased from 0.50 (0.29) µm to 0.42 (0.14) µm ($P<0.05$). Whereas, median (IQR) Sa of polished enamel significantly increased for all three erosion times from 0.08 (0.10) µm to 0.26 (0.02) µm ($p<0.001$) (15 minutes), 0.15 (0.11) µm to 0.25 (0.07) µm ($p<0.001$) (30 minutes) and 0.10 (0.08) µm to 0.27 (0.04) µm ($p<0.001$) (45 minutes). Finally, the *in situ* study demonstrated no significant changes in Sa roughness of natural unpolished enamel regardless of either erosion time or whether exposure was *ex vivo* or *in vivo*. However, mean (SD) Sa roughness of polished enamel significantly increased for all three erosion times from 0.04 (0.01) µm to 0.09 (0.03) µm ($p<0.05$) (15 minutes *ex vivo*), 0.04 (0.01) µm to 0.12 (0.04) µm ($p<0.05$) (30 minutes *ex vivo*), 0.04 (0.01) µm to 0.13 (0.04) µm ($p<0.05$) (45 minutes *ex vivo*), 0.04 (0.02) µm to 0.08 (0.04 µm) ($p<0.05$) (15 minutes *in vivo*), 0.04 (0.01) µm to 0.10 (0.04) µm ($p<0.05$) (30 minutes *in vivo*) and 0.04 (0.01) µm to 0.07 (0.03) µm ($p<0.05$) (45 minutes *in vivo*).

By optimising the measurement protocol in the early part of the thesis 3D (Sa) enamel surface texture was quantified over different locations of the natural unpolished enamel. This confirmed that the roughness of the central area of unpolished enamel samples was representative of the overall sample which allowed reliable measurements from this area in future studies. This central area of the samples represented the apex of curvature and thus provided the least data drop out from the optical scanners and was also in the region of more homogenous natural prism morphology. The *in vitro* investigation of the different erosion times identified that not only was natural unpolished enamel more resistant to erosion than polished samples but moreover natural enamel behaved differently to polished enamel by becoming smoother following erosion rather than rougher. The protocol developed for the *in situ* study successfully provided further confirmation of the resistance of unpolished natural enamel

when the natural inhibitory effects of the oral environment were combined. This demonstrated the positive effects of natural resistance to erosive tooth wear.

Therefore, this thesis has developed a method for quantifying 3D surface roughness changes occurring in natural unpolished enamel and polished enamel during erosion, which revealed significant complexity in the surface texture response of natural unpolished enamel to a dietary erosive challenge.

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Acknowledgements

Professor David Bartlett my first supervisor, thank you I am eternally grateful to have been given this opportunity. You have provided care, guidance and overwhelming support throughout this journey. You look after all of us like a member of the family and we are extremely fortunate to have you as our supervisor.

Dr Rupert Austin, my second supervisor you have always provided positive support and encouraged me in every way. Your optimism and positive attitude to research provides motivation even when faced with negative results.

Dr Rebecca Moazzez (my adopted supervisor) your kind words and suggestions have always been appreciated.

Dr Charles Parkinson, Dr Steve Mason and Mr Shaz Khan at GlaxoSmithKline. GSK provided not just funding for this PhD but advice, support, training and use of equipment.

To my colleague and friend Dr Ryan Olley for giving me an opportunity for publication and personal support throughout the writing of this thesis.

Messrs Peter Pilecki and Richard Mallett, thank you for all your help and support in the laboratory.

To my PhD colleagues Miten Mistry, Mahdi Mutahar, Saoirse O'Toole and Petros Mylonas. Thank you for friendship, support, encouragement and feedback on my thesis.

This thesis would not have been possible if it were not for the kind staff in the Oral Surgery Department at Guy's Dental Hospital. Thank you to the consultants for allowing me to approach their patients to gain consent for the collection of extracted teeth. Thank you to all the health care staff for all your assistance.

Thank you to the Oral Clinical Research Unit, Mr Daniel Abrahams, Mrs Rz Dakri, Mrs Jagrul Miah and Mrs Linda Gane for making the Clinical Trial run so smoothly.

Finally, thank you to my parents who have always encouraged and supported me in all my choices. You have taught me to work hard, have faith in myself and to be independent. You have shown me that perseverance and endurance are worthwhile. I am proud to have you as parents and hope to justify the faith you have in me.

Chapter 1 Literature review

1.1 Enamel Erosive Tooth Wear

1.1.1 Structure of natural human enamel

Tooth wear is described as the irreversible loss of dental hard tissues from mechanisms other than bacteria or trauma (Bartlett & Smith 2000). The outer structure of a tooth is enamel, which is a highly mineralised hard tissue. Human enamel is the hardest biological mineralised tissue and is constituted of 96 % mineral, 3 % water and 1 % organic protein (Cuy et al. 2002). The mineral content is composed of a calcium deficient carbonated hydroxyapatite (HA) (Ca10-x Nax (PO4)6-y (CO3)z (OH)2-u Fu) (Featherstone & Lussi 2006). The term calcium deficient hydroxyapatite refers to natural substitution that occurs between the crystallites and the oral environment, whereby calcium ions are often replaced by other metal ions (sodium) and hydroxide ions (OH) can be replaced by fluoride (Featherstone & Lussi 2006). At the nanostructured level, enamel HA crystallites combine to form hexagonal 3D prisms or rods, each approximately 3-6 µm in diameter, which are separated by an organic protein complex which provides enamel with its strength and excellent resistance to crack propagation (West & Joiner 2014; Cuy et al. 2002). However, within this ultrastructure the orientation of these crystallites is not entirely uniform, with the greatest variation identified at the occlusal surface where function determines the strength and durability (Simmons et al. 2011; Al-Jawad et al. 2007). The orientation and alignment of the prisms themselves are also highly variable therefore in different locations within the enamel the micro-structure varies: at the dentinal enamel junction (DEJ) the prisms are randomly orientated, in outer enamel the prisms mostly run longitudinally and emerge perpendicular to the occlusal plane, whereas in cuspal areas the prisms can cross each other resulting in decussation patterns, (West & Joiner 2014; Hirota 1982; Raue et al. 2012; Braly et al. 2007).

It has been postulated that this may influence measurement of surface texture during tooth wear, as regions of prism decussation and random alignment of prisms result in a more highly textured surface than regions with uniform prism alignment, as seen in outer enamel. However, the structure of outer

enamel is more complex than prisms simply emerging perpendicular to the outer enamel surface. Indeed, scanning electron microscopy (SEM) studies reveal that outer enamel contains areas devoid of prisms (aprismatic enamel); in these areas the prism junctions and orientation are often highly complex and no clear prism structure is identifiable (Whittaker 1982). Whittaker (1982) identified 3 types of surface enamel prism structure: type 1 whereby prisms were visible at the tooth surface, type 2 no prisms visible and type 3 a complex arrangement showing a combination between type 2 and type 3. Therefore, whilst the phrase aprismatic is used, it does not literally mean that there are no prisms present, merely that they are not easily identified. This has significance when investigating changes in the enamel surface caused by erosive tooth wear. Meurman and Frank (1991) used SEM to investigate changes in the surface of natural human enamel following an erosive challenge identifying a variation over different locations in the surface. Many erosive wear research studies use enamel samples which have been polished flat, a process which removes the natural outer layer containing areas of aprismatic enamel which has the effect of making the erosive surface changes more uniformed and pronounced (Meurman & Frank 1991; Ganss et al. 2000). In contrast, the complex structure in the aprismatic enamel results in innate resistance to erosion, and therefore to fully understand the process of erosive tooth wear investigations must be carried out using natural unpolished enamel surfaces which are considered more clinically relevant than polished enamel.

Further to aprismatic enamel, there are other microstructural considerations in enamel with relevance to surface texture measurement including Retzius lines and prism cross striations linked to the amelogenesis process. The Retzius lines result from the position of the ameloblast layer at various points of time during enamel development and are both part of natural development and a stress response whereas prism cross striations are thought to be part of the normal rhythms of development but can also be induced by acid (Li & Risnes 2004) which are shown in Figure 1.

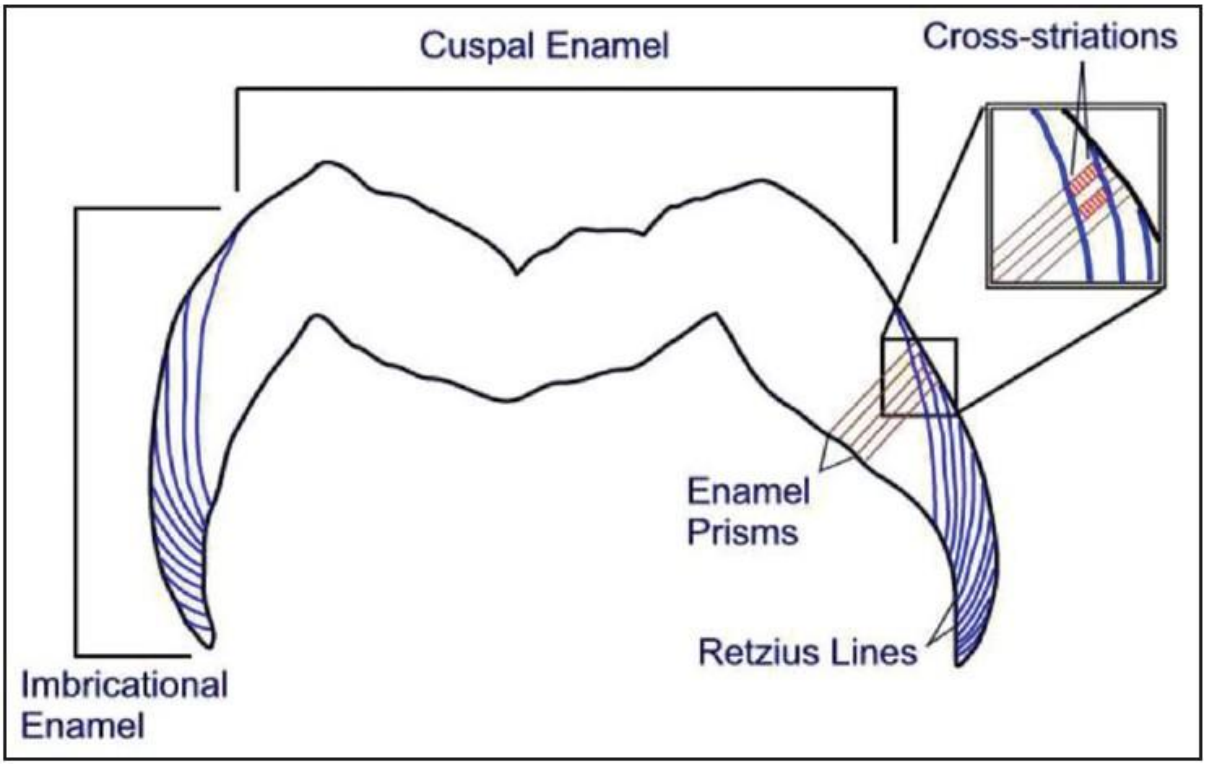


Figure 1: Schematic showing the arrangement of enamel prisms projecting the DEJ to the outer surface of the enamel and the presence of Retzius lines and cross striations (Bharanidharan et al. 2014).

The variation in the structure of enamel has been investigated in relation to mechanical properties (Carvalho & Lussi 2015). There is debate to whether prism orientation affects the mechanical strength of enamel or whether this is linked to the variation in calcium (CaO) and phosphate (P_2O_5) chemistry. However, overall variation in chemistry is the main contributing factor, which is important for studies of erosive tooth wear as during the erosive process the underlying chemistry is altered. Originally studies investigated the variation in mechanical properties linked to physical structural variation. Staines et al. (1981) investigated the elastic properties of enamel over different locations relating to different prism orientation and concluded that variations in elasticity occurred due to moisture content and prism orientation. Cuy et al. (2002) took this further in a study which used nanoindentation to investigate human enamel and correlated the variation in mechanical properties with differences in chemical content and microstructure across different surface locations and depths. They reported that the enamel became gradually softer from the outer surface to the DEJ and this

correlated strongly with decreases in decreases in phosphate (P_2O_5) and calcium hydroxide (CaO) concentration, however, they identified only a weak correlation with prism orientation. This study was limited as only three teeth were investigated. However, a study by Braly et al. (2007) further investigated the relationship between prism orientation and mechanical properties of enamel. They minimised the impact of chemical variation by investigating mutually perpendicular surfaces near their common edge over a very small volume of enamel ($\sim$0.1 mm^3) therefore leaving prism orientation as the only variant. They performed nanoindentation on the cross-sectional cut surface in arrays that ran towards the occlusal surface away from the DEJ and on the occlusal surface in arrays that ran perpendicularly towards the cross-sectional cut surface. However, they observed no significant difference in the mechanical properties when measuring perpendicular or parallel to the prisms, thus suggesting that chemical variation had more influence over mechanical properties rather than prism orientation. Habelitz et al. (2001) attributed differences in mechanical properties between interprismatic enamel and enamel prisms to the difference in organic content, as interprismatic enamel has a higher organic component. Kodaka et al. (1998) combined hardness testing and Raman Spectrometry to investigate the relationship between the mineral content and mechanical properties of enamel. They identified a correlation between increased mineral content and hardness. This has significance for erosive tooth wear, and the susceptibility of individuals to wear. Carvalho and Lussi (2015) investigated the effects of erosion on calcium loss and surface hardness at different enamel depths. Whilst they identified that surface hardness was significantly softer towards the DEJ, there was no statistical difference in hardness change. Therefore, the mechanical variation does not affect response to erosion. However, when calcium loss was measured there was a correlation with enamel depth, with significant reduction in calcium loss observed towards the DEJ, where the natural composition would have less calcium content to start off with. Therefore, if calcium loss is to be an outcome measurement of erosive tooth wear some concept of the original composition of the samples is required to generate a balanced ratio of the effect of what has been lost. If enamel samples are to

be polished flat for erosion studies this must be done methodically in accordance with strict protocols to aim towards a standardised enamel depth.

1.1.2 The evolution of concepts of erosive tooth wear

There has been a change in perception and understanding of tooth wear with a move towards the term 'erosive tooth wear' which has a modified and distinct meaning (Lussi & Carvalho 2014). Tooth wear was the blanket term previously used. Tooth wear is not a modern phenomenon and within anthropological circles it has been considered a normal physiological process. However, anthropological studies do not include a distinction between the mechanisms of wear and in these studies it tends to be focused on abrasive wear from diets (Kaidonis 2007). Interestingly, a historical decrease in tooth wear has been noted as diets changed becoming softer (Kaifu 1999). Despite tooth wear historically being considered a normal physiological process there is now a move towards classifying tooth wear as pathological in nature (d'Incau et al. 2012) and this led Smith and Knight (1984) to make a distinction between physiological wear and pathological wear. They proposed the concept of pathological wear to be a loss of function, serious aesthetic deterioration and a balance between whether 'the tooth will survive the rate of wear'. Pain is another consideration which can alter the perception from a physiological to pathological condition. Severe erosive tooth wear can be associated with dentinal hypersensitivity and endodontic complications (Ganss 2014). Therefore, there is no denying that the classification of tooth wear as a pathological oral disease is more appropriate than the previous concepts of it being a natural physiological process.

Modern dental experts consider that tooth wear occurs from a combination of attrition, abrasion and erosion and there is a clinical consensus over these definitions. Erosion is the chemical dissolution of tooth structure by acids other than those caused by bacteria. Attrition and abrasion are both mechanical wear, attrition by tooth to tooth contact and abrasion by foreign objects such as tooth brushing (Kaidonis 2007; Shellis & Addy 2014). Clinically, it can be difficult to differentiate the cause of the wear, and most often it is due to a combination of two or more of these elements. For this

reason tooth wear has acted as a blanket term for all clinical evidence of all causes of tooth wear (Smith et al. 1997; Bartlett 2005). However, this is now outdated and 'erosive tooth wear' is deemed a more appropriate terminology.

Bartlett (2005) stated that the most common presentation of tooth wear results from a combination of erosion and abrasion. The outer layers of tooth tissue (3-5 µm) are weakened by acid challenge which increases the susceptibility of the enamel and dentine to abrasion from tooth brushing with or without toothpaste (Bartlett 2005). Furthermore, Addy and Hunter (2003) concluded that abrasion with toothpaste would only occur in an acidic environment with the critical pH being < 5.5. Lussi and Carvalho (2014) argued that there was no fixed 'critical pH' for erosion, and that it was dependent upon mineral content of solutions. However, they still recognised that an acidic environment was required for abrasive tooth wear to occur. There has been a move towards referring to tooth wear as erosive tooth wear in recognition of the influence of erosion in generalised tooth wear (Bartlett 2016).

Therefore, due to this increased awareness of erosion being the most important aetiological factor, the term 'erosive tooth wear' has now superseded previous terms such as 'tooth wear' and 'tooth surface loss'. A UK study in 2003 identified that only 36 % of the primary care dentists who took part regularly noticed the presence of erosive tooth wear (Dugmore & Rock 2003). However, dentists are becoming more clinically aware of erosive tooth wear and new screening indices such as the Basic Erosive Wear Examination have been developed (Bartlett & Dugmore 2008; Mehta et al. 2012; Olley et al. 2014; Lussi & Carvalho 2014). Lussi and Carvalho (2014) suggested that the increased awareness and clinical responsibility towards erosive tooth wear were directly linked to the global increases in consumption of acidic food and drinks and the increase in prevalence of erosive tooth wear. Their review identified a sharp rise in the number of studies investigating erosive tooth wear; with an electronic medical database search revealing less than 5 studies published in 1970, 10 in 1980 to almost 60 in 2000 and 100 studies published in 2012.

Bartlett et al. (2013) also established a correlation between increased severity of erosive tooth wear and increased consumption of acidic products. Awareness of the general public to erosive tooth wear was investigated in a cross-sectional epidemiological study to define attitudes and awareness of erosion in 18 year olds in Norway. Participants completed a structured questionnaire to assess their awareness and attitudes towards dental erosion and were also clinically examined for erosion; 88% had heard of damage to teeth by erosion, 78% of all participants stated that they would be concerned if their teeth were damaged by acids and 84% stated that they would change their habits if such damage had occurred. The proportions of participants who were concerned and ready to change their habits were significantly higher among participants without erosive tooth wear (Skudutyte-Rysstad et al. 2013). This suggests that although awareness is increasing the message of the importance of erosive tooth wear is not reaching its target audience, indeed this has been identified as a potential public health issue whereby those who are potentially at greatest need of prevention are perhaps least likely to present requesting advice about their tooth wear (Burt 2005).

1.1.3 Prevalence of erosive tooth wear

Epidemiological data suggests that tooth wear increases with age and over the recent decades there is a suspicion that the prevalence of tooth wear has increased in developed countries (Van'T Spijker et al. 2009; White et al. 2012). It has also been suggested the prevalence of severe tooth wear is higher in the UK than other countries, therefore it is imperative to identify early erosive changes to improve early diagnosis and prevention of this pathological oral disease (Bartlett et al. 2013). This change in pattern of tooth wear is linked to wider developments such as a change in diet from rougher to softer foods has resulted in less abrasive wear than that recorded in anthropological studies, in contrast to more recent times where increased consumption of acidic products is thought to contribute to increases in erosive tooth wear (Kaifu 1999; Lussi & Carvalho 2014).

Erosive tooth wear is not only a concern for adults where traditionally it would be expected for some wear to be present, it is increasingly an issue also affecting the younger populations. Nunn et al. (2003)

identified an increase in the prevalence of erosive tooth wear in children between studies carried out in 1993 and 1996/7. Salas et al. (2015) conducted a systematic review, which investigated the prevalence of erosive tooth wear present in the permanent dentition of children and adolescents in the Americas, Europe, Asia and the Middle East estimating the overall prevalence at 30.4%. One of the difficulties when comparing different epidemiological studies is the variation in indices used. This results in challenges in comparing the severity scores between different studies and will be discussed more in section 1.1.5 below. A Europe-wide study by Bartlett et al. (2013) indicated that the UK exhibited higher prevalence of moderate scores for tooth wear. The Adult Dental Health Survey in 2009 suggested that 77 % of dentate adults experienced tooth wear on their upper incisors (White et al. 2012). The report acknowledged the cumulative effect of tooth wear with age and suggested that mild to moderate tooth wear would be expected in an older population but raised the issue of public health concern in younger populations exhibiting moderate to severe wear. Again, this highlights the transition of tooth wear as a natural physiological phenomenon to the pathological disease of erosive tooth wear and the need for early clinical diagnosis.

Understandably there has been a recent interest in examining tooth wear in younger age groups. Isaksson et al. (2014) conducted a study to investigate the prevalence of dental erosion and associated habits in 20 year olds in Sweden using data corroborated from an examination and interview in 2007. The interview recorded consumption of dietary acids including fruit-based and carbonated beverages, two scores were used to visually assess the severity of erosive wear. In total, 494 individuals took part in the study, 75 % of which showed signs of erosion, however the presence of severe erosion was low at 2 %. Unsurprisingly, there was a correlation between increased consumption of soft drinks and individuals with extensive erosion than in those with no erosion. They also identified links between erosion, oral health and body mass index (BMI). Participants classified as overweight or obese were more likely to have more erosion identified and caries rates also tended to be statistically higher in individuals who also had erosive tooth wear (Isaksson et al. 2014). There has also been some suggestion that in children the presence of erosive tooth wear may be linked to socioeconomic

background, with higher prevalence of those in lower socioeconomic backgrounds, and a North-South divide within the UK (Nunn et al. 2003; Al-Dlaigan et al. 2001). Therefore, within the younger population erosive tooth wear may be part of an overall public health issue and collaboration between oral health providers and medical health providers is needed to direct the correct health advice to those most at risk.

1.1.4 Clinical presentation and diagnosis

The clinical diagnosis of erosive tooth wear still relies upon a visual examination (Bardsley 2008; Huysmans et al. 2011; Ganss & Lussi 2014). Therefore, early erosive changes are notoriously difficult to detect. Patients are unlikely to detect the smooth shiny appearance of enamel themselves and therefore will seek treatment late when irreversible tooth surface loss has occurred (Amaechi & Higham 2005). Bartlett (2005) explained how the clinical appearance from the three mechanisms of tooth wear varies. Attrition results in flattened occlusal surfaces with equal wear in both arches and might be associated with hypertrophic masseter muscles, particularly with bruxism. Erosion results in a general loss of enamel with a smooth shiny appearance particularly on the buccal and lingual surfaces of upper incisors. Abrasion acting independently is considered rare. When erosion and attrition are combined, there is 'cupping' on the occlusal surfaces of the molars and premolars and the incisal surface of the anterior teeth as shown in Figure 2.

Figure 2: Image showing typical features of erosive tooth wear including the smooth shiny appearance of enamel, the exposure of the underlying dentine and the cupping effect on the cuspal regions.

1.1.5 Tooth wear indices

Currently clinical diagnosis and monitoring of erosive tooth wear relies on indices. Tooth wear indices are also widely used in research studies, with many different indices developed throughout the world leading to confusion and difficulties in comparing studies (Bardsley 2008). One of the earliest versions of a tooth wear index was used in a study investigating the effects of erosion of animal teeth (Restarski et al. 1945). The first clinical tooth wear index was developed by Eccles (1979) shown in Table 1. This differentiated between different causes of tooth and provided a basis for the development of future indices. Smith and Knight (1984) developed the Tooth Wear Index (TWI), which was a more comprehensive system. The TWI recorded evidence of wear for each surface (buccal, lingual, distal, mesial) of all teeth present reporting the severity shown in Table 2 (Smith & Knight 1984). However, it did not distinguish between the aetiology. Whilst a wide variety of indices have been developed the TWI remains one of the most common used in research studies. There have also been indices developed specifically for identifying erosive tooth wear in children (O'Sullivan 2000; Restarski et al. 1945). In 2008 an inter-European collaboration attempted to bridge the gap between a research tool and a clinical diagnostic aide. Bartlett et al. (2008) proposed the BEWE index as a screening tool, based

on the BPE (Basic Periodontal Examination). When using the BEWE, the dentition is divided into sextants and each sextant is given a score representing the most severely worn surface in that sextant. The score is dependent upon the level of erosive tooth wear noted with 0 meaning no erosion, 1 initial loss of surface texture, 2 distinct defect/hard tissue loss affecting less than 50 % of the tooth surface area and 3 hard tissue loss equal to or more than 50 % of the surface area, as shown in Table 3. A cumulative score can be calculated from all sextants and linked to clinical management recommended by the authors, as shown below.

Table 1: Eccles tooth wear index (Eccles 1979).

Class	Surface	Criteria
Class I		Early stages of erosion, absence of developmental ridges, smooth, glazed surface occurring mainly on labial surfaces of maxillary incisors and canines
Class II	Facial	Dentine involved for less than one third surface; two types
		Type 1 (commonest): ovoid–crescentic in outline, concave in cross section at cervical region of surface. Must differentiate from wedge shaped abrasion lesions
		Type 2: irregular lesion entirely within crown. Punched out appearance, where enamel is absent from floor
Class IIIa	Facial	More extensive destruction of dentine, affecting anterior teeth particularly. Majority of lesions affect a large part of the surface, but some are localised and hollowed out
Class IIIb	Lingual or palatal	Dentine eroded for more than one third of the surface area. Gingival and proximal enamel margins have white, etched appearance. Incisal edges translucent due to loss of dentine. Dentine is smooth and anteriorly is flat or hollowed out, often extending into secondary dentine
Class IIIc	Incisal or occlusal	Surfaces involved into dentine, appearing flattened or with cupping. Incisal edges appear translucent due to undermined enamel; restorations are raised above surrounding tooth surface
Class IIId	All	Severely affected teeth, where both labial and lingual surfaces are extensively involved. Proximal surfaces may be affected; teeth are shortened

Table 2: TWI tooth wear index developed by Smith and Knight (Smith & Knight 1984).

Score	Surface	Criteria
0	B/L/O/I	No loss of enamel surface characteristics
	C	No loss of contour
1	B/L/O/I	Loss of enamel surface characteristics
	C	Minimal loss of contour
2	B/L/O	Loss of enamel exposing dentine for less than one third of surface
	I	Loss of enamel just exposing dentine
	C	Defect less than 1 mm deep
3	B/L/O	Loss of enamel exposing dentine for more than one third of surface
	I	Loss of enamel and substantial loss of dentine
	C	Defect less than 1–2 mm deep
4	B/L/O	Complete enamel loss–pulp exposure–secondary dentine exposure
	I	Pulp exposure or exposure of secondary dentine
	C	Defect more than 2 mm deep–pulp exposure–secondary dentine exposure

Table 3: BEWE screening index (Bartlett et al. 2008).

Score	Criteria
0	No erosive tooth wear
1	Initial loss of surface texture
2	Distinct defect, hard tissue loss < 50 % of surface area
3	Hard tissue loss > 50 % of surface area

Table 4: BEWE cumulative score and treatment advice (Bartlett et al. 2008).

Risk factor	Cumulative score	Clinical management
None	Less than or equal to 2	Routine maintenance and observation Repeat at 3-year intervals
Low	Between 3 and 8	Oral hygiene and dietary assessment, and advice, routine maintenance and observation Repeat at 2-year intervals
Medium	Between 9 and 13	Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces Ideally, avoid the placement of restorations and monitor erosive wear with study casts, photographs, or silicone impressions Repeat at 6–12-month intervals
High	14 and over	Oral hygiene and dietary assessment, and advice, identify the main aetiological factor(s) for tissue loss and develop strategies to eliminate respective impacts Consider fluoridation measures or other strategies to increase the resistance of tooth surfaces Ideally, avoid restorations and monitor tooth wear with study casts, photographs, or silicone impressions Especially in cases of severe progression consider special care that may involve restorations Repeat at 6–12-month intervals

Any clinical examination which solely relies upon visual examination by a clinician can be criticised for inter operator discrepancies and when used for research studies inter and intra operator calibration must first be carried out (Dixon et al. 2012). Olley et al. (2014) validated the BEWE as an effective representation of the full clinical presentation of erosive tooth wear. In their study, one examiner was trained and calibrated by recording a BEWE score for each of 90 tooth surfaces provided in a power point presentation and the scores compared to those of an expert gold standard examiner. Once calibrated the operator compared cumulative BEWE scores, BEWE calculations of percentage of tooth surface affected and highest BEWE score per sextant for 350 participants finding a good correlation amongst all three. Despite this supportive data, there remains concerns regarding the limitations of the BEWE when scoring localised advanced tooth wear, as this could produce a cumulative score suggesting low risk despite there being an isolated area of severe dentine exposure. Moreover, the BEWE does not take into account the age of the patient and whilst there have been advances in the use of screening tools there remain many unresolved issues with early detection of erosive tooth wear. In addition, the BEWE is less clear when discriminating between the earliest stages of tooth wear, especially the score 1 which is loosely defined as initial loss of surface texture (Dixon et al. 2012). However, the original concept of the BEWE was as a screening tool, and like the BPE, there remains a crucial role to record tooth wear in a practice setting.

To understand progression particularly in the early stages of erosive tooth wear methods to visualise and quantify the surface change must be used. Bartlett (2016) points out that the initial surface texture loss, the first sign of erosive tooth wear according to the BEWE, is yet to be clearly defined. This remains a clinical challenge. It is also worth considering that the early stages of erosive tooth wear may be more susceptible to the preventive action of fluorides and other agents and yet despite this there is no reliable method to measure any change *in vivo*.

1.2 Methodologies to investigate erosive tooth wear

1.2.1 Sample preparation

1.2.1.1 Bovine versus human enamel

Both bovine and human teeth are commonly used in erosion studies. Researchers argue that bovine enamel is easier to source making its usage easier to facilitate. Certainly, the Human Tissue Act does exert restrictions on the usage of human enamel for research. UK legislation in the Human Tissue Act includes human teeth as a body part, therefore ethical approval and informed consent are required to use them in any research study. Other practicalities which are pertinent for research studies indude the physical size of bovine teeth meaning that more sections can be prepared from one tooth, diet and exposure to fluoride can be more standardised compared to human donations. However, their environmental exposures and structure are different to human enamel (Laurance-Young et al. 2011). Meurman and Frank (1991) investigated the effects of erosion on human and bovine enamel using SEM. Coca Cola was used as the erosive agent for both types of sample and they were immersed for 15, 30, 60, 120 or 180 minutes. The authors reported similarities in response to erosion from bovine enamel and human enamel. However, this study was limited as it relied solely on qualitative analysis of the SEM images. Quantitative studies suggest that whilst there are visual similarities in the erosion response there may be significant differences in structural changes. Amaechi et al. (1999) compared the response to erosion of bovine permanent enamel, permanent human enamel and deciduous human enamel. Ten samples were prepared from each tooth type and polished using a pumice and 1200 grit which underwent erosion cycling for 24 days, nail varnish was used to create a window of exposed enamel. Following erosion cycling sectioning and microradiography were conducted to measure the depth of the erosion lesions. They identified a significant difference between permanent human enamel and deciduous human enamel and between permanent human enamel and permanent bovine enamel. There were significantly smaller erosion depths for permanent human enamel. They also identified that human deciduous enamel demonstrated significantly lower depths

compared to permanent bovine enamel. Furthermore, in an *in situ* study which used in an *ex vivo* erosion-abrasion regime, Rios et al. (2006) compared the effects on bovine and human enamel. Contact profilometry was used to assess erosion depths and microhardness before and after the erosion and they expressed microhardness as percent surface microhardness change (%SMC) calculated as the percentage related to initial hardness. Bovine enamel demonstrated significantly lower %SMC, meaning it became significantly softer compared to human enamel with significantly deeper wear lesions. Attin et al. (2007) investigated the use of permanent and deciduous bovine enamel as a substitute for human permanent and deciduous enamel in wear studies. They found no difference between substrates for abrasion only, however for erosion only and erosion abrasion bovine enamel demonstrated significantly greater tissue loss compared to both human deciduous and permanent enamel. In a study comparing the response of bovine and human enamel to single drink conditions of erosion, White et al. (2010) suggested that bovine enamel is 30% more responsive to erosion than human enamel but concluded that as long as this is interpreted in results bovine enamel remains a suitable test substrate. However, this should be disputed and whilst bovine enamel is widely used it is not a representative substrate. Therefore it can be concluded that human enamel is the optimal substrate to effectively investigate the effects of erosive tooth wear.

1.2.1.2 Tooth type and tooth side

After deciding upon human or bovine enamel further considerations are necessary; natural unpolished or polished samples, tooth type and even tooth side. In an early study, Sullivan (1954) investigated the solubility of polished and natural unpolished enamel suggesting that natural unpolished enamel was less soluble after initial dissolution stabilisation had occurred, which was not seen in the polished enamel. Meurman and Frank (1991) compared the effects of dietary erosion on polished and natural unpolished bovine enamel with SEM imaging. They identified that the polished samples were more susceptible to erosion than the natural unpolished samples suggesting that the outer layer of enamel, which is removed during polishing, is naturally more resistant to erosion. Ganss et al. (2000)

investigated the effects of erosion with citric acid on polished and natural human enamel using contact profilometry to measure tissue loss. The natural unpolished and polished samples were prepared from the same extracted molars, each used to produce a total of eight enamel samples. The natural unpolished enamel samples demonstrated significantly less tissue loss than the polished enamel again suggesting that the outer surface of enamel is more resistant. Hara et al. (2016) investigated changes in surface texture of natural unpolished and polished human enamel following the same erosion procedure. When using the roughness parameter Sa (Surface Roughness Average) they were unable to identify surface roughness changes of natural unpolished enamel following erosion but were able to identify changes in the polished enamel samples, again this suggests that natural unpolished enamel is less susceptible to erosion than polished enamel. Despite these apparent differences in susceptibility it is polished samples that remain the most commonly used substrate as they provide optimum surfaces for gold standard measurements such as profilometry and microhardness testing. However, polished enamel samples cannot be recognised as clinically representative, therefore there remain gaps where methods to identify erosive changes on natural unpolished enamel must be developed.

A further consideration with regards to the appropriate substrate is to compare tooth side and tooth type. Tucker et al. (1998) used calcium analysis to determine the solubility of buccal and lingual/palatal regions of incisors, canines, premolars and molars from both maxillary and mandibular arches and suggested that the lingual/palatal surfaces were more susceptible to erosion for both arches, with maxillary teeth more susceptible to erosion than mandibular teeth. Carvalho and Lussi (2015) compared microhardness change and calcium release of premolars and molars at different enamel depths, furthermore investigating the difference between buccal and lingual samples from the same tooth type. They identified that calcium loss and microhardness change were different in molars compared to premolars, but there was no difference in microhardness change of different sides of the same tooth type. With regards to enamel depth they identified that in both tooth types more calcium was released in the deeper layers, however there was no significant difference in microhardness

change. Therefore, they suggested when calcium release is used to quantify erosion the polishing regime should be standardised to ensure the same enamel depth across as the samples being investigated. Mistry et al. (2015) compared tissue loss and microhardness change of polished buccal and lingual enamel samples from human premolars and molars. Their results suggested no difference between tooth types with regards to tissue loss as measured by step height. However, there was a difference in microhardness change between molars and premolars with significantly greater microhardness change identified for premolars. When comparing tooth sides of the same tooth type both tissue loss and microhardness change were significantly different. The contrasting results for the difference in microhardness change of different sides of the same tooth type may be due to other experimental variants. Carvalho and Lussi (2015) used a maximum erosion time of 3 minutes in citric acid whereas Mistry et al. (2015) chose 50 minutes in citric acid and microhardness testing becomes less accurate with more erosion (Schlueter et al. 2011). However, the erosion regime chosen by Mistry et al. (2015) was optimum for measuring step height loss and their results concerning this measurement strongly indicated that using both tooth sides may influence results. Overall, it is likely that using different tooth sides and types may skew results. Therefore, only one type (preferably molars) and only one side (buccal) should be used, unless for a specific reason such as trying to identify surfaces with less natural curvature for natural unpolished samples.

1.2.1.3 Smear layer

A smear layer is a layer of organic and inorganic debris which is formed when a hard tissue is cut by hand or rotary instruments (Pashley 1992). Smear layers are most commonly associated with endodontics where removal is carried out using chemical agents including citric acid (Salama & Abdelmegid 1994). Within erosion studies the smear layer is created during the sample preparation and can influence the measurement of enamel surface changes during erosion studies. Hughes et al. (1999b) identified enamel tissue loss of 0.1 µm after five days for the control polished enamel samples in their *in situ* study (no exposure to acid, only water). They suggested that this was potentially related

to the initial presence of a smear layer which was removed after the five days. A smear layer would have been created following the sectioning of the teeth and polishing samples flat with carbide grits as part of the sample preparation. Watari (2005) estimated the smear layer of enamel samples which had been polished flat to a 2000 grit to be 270 nm thick. When reference barriers are used to cover part of the sample leaving a window of enamel that will be exposed to acid, the acid will have removed the smear layer as well as induced surface alteration of the underlying enamel, whereas the reference areas (which were covered during the erosion cycling) would still be covered with the 270 nm smear layer. This results in over estimation of the depth of the erosion lesion (Mistry et al. 2015). Bortolotto et al. (2009) investigated smear layers created on enamel and dentine from different cavity preparations. They identified that the smear layers created for enamel were consistently thicker than those for dentine prepared in the same way. Therefore, if producing dentine and enamel samples for the same study it must be ensured that the smear layer is effectively removed. Salama et al. (1994) investigated the efficiency of different chemical solutions including citric acid and hydrogen peroxide at removing endodontic smear layers. They identified that 6 % citric acid successfully removed the smear layer after 15 seconds suggesting solubility in acid. These were from qualitative observations and therefore are limited. Moreover, for erosion studies where the effect of acid on the topography and mechanical properties of tooth tissue is being investigated any solution that could alter these would not be appropriate. Sanches et al. (2009) investigated the topographical effects on polished bovine enamel and dentine samples caused by removing the smear layer with lactic acid solution. Samples were allocated into four experimental groups, with groups 1 to 3 etched with lactic acid for 1, 3 and 5 minutes and the final group a control with no acid exposure. They identified that after only one minute of etching the smear layer was removed and the enamel topography was altered. Therefore, within erosion studies ultrasonication is the gold standard to effectively remove the smear layer using mechanical means and not causing alteration to the enamel or dentine structure and was used in this thesis. Ultrasonication can also be used to quantify erosive changes, as it has been shown to remove the softened enamel left behind following erosion (Barbour & Rees 2004). *In vitro,* the

presence of a smear layer is an unwanted effect from the sample preparations process that must be removed. However, the smear layer is thought to have a beneficiary effect clinically for erosive tooth wear. In areas of clinical abrasion where the enamel has worn away and the underlying dentine is exposed and the presence of a smear layer has been identified microscopically. Interestingly these areas are not normally associated with pain or dentinal hypersensitivity suggesting the smear layer provides a natural therapeutic effect (Kaidonis 2007).

1.2.2 Storage of enamel samples

There are differences in how prepared enamel samples are stored between different erosion research groups. Eisenburger et al. (2001b) stored their test samples in artificial saliva and control samples in saline for 24 hours prior to commencement of the experiment as part of a demineralising-remineralising regime. Whilst storing samples in saline or distilled water for 24 hours is unlikely to affect samples caution must be taken if there is a lengthy delay between preparation and the next stages of a study as the pH of the storage solutions may change over that time and alter the surface of the samples (Mistry et al. 2015). Cuy et al. (2002) advocated storing enamel samples under ambient conditions as they believed that drying of the samples would affect their elasticity and hardness properties. However, further work disproved this theory. Attin et al. (2009) investigated the effect of storing enamel in wet or under ambient conditions (21°C, 35 % air humidity) by taking repeated profilometric measurements using a contact profilometer. The same samples were used for both parts of the experiments initially stored wet with profilometric tracings being recorded whilst the samples remained submerged in water followed by the repeated measurements whilst the samples were kept in ambient conditions, without measures conducted to dry out the samples first. There were no significant differences in the measurements between the two methods of storage, suggesting that enamel samples remain stable despite drying out therefore and so there is no necessity for enamel samples to be stored wet. Furthermore Mistry et al. (2015) compared storage of samples prior to erosion cycling and measured the outcome using microhardness change and profilometry. They

investigated the effect of storing the samples dry by allowing them to air dry over a 24 hour period and storing them in deionised water after 1 hour or 24 hours measuring microhardness change and tissue loss with an optical profilometer. They reported significant differences for both measurements with air dried samples exhibiting significantly greater tissue loss and microhardness changes compared to samples stored in deionised water for either 1 hour or 24 hours. However, the authors recommended storing enamel samples dry for convenience and consistency. This is a reasonable suggestion as the pH of deionised water will alter over time and the addition of buffering agents during storage may affect experimental outcomes. Mullan et al. (2017b) identified the level of shrinkage which occurs in enamel following dehydration and rehydration which was a negligible 0.03%. Overall the choice of storage for the samples is the question of what effects the dehydration will have to the enamel properties. From the evidence discussed, there is no need to store enamel samples wet and the risk of pH changes of the solution outweighs any preconceived benefit (Mistry et al. 2015, Mullan et al. 2017b). Enamel samples should optimally be stored dry, however this not a true simulation of the oral environment. Therefore, following storage of the enamel samples the effects of saliva and the salivary pellicle should be considered. These can either be introduced by *in vitro* or *in situ* methods, which will be discussed in section 1.3.1 below.

1.2.3 *In vitro* erosion regimes

Chemical erosion of enamel occurs either by the hydrogen ion derived from acids or chelating agents in which anions bind or complex calcium (Featherstone & Lussi 2006). Most commonly it is acids which are the origin of erosive tooth wear. These acids can be intrinsic, hydrochloric acid from gastric juices or extrinsic sources including food, drinks and medications. Common dietary extrinsic acids include; acetic acid sourced in vinegar, ascorbic acid vitamin C found in citrus fruit, citric acid found in citrus fruit, lactic acid in dairy products, malic acid found in apples and tartaric acid found in grapes and finally phosphoric acid is found in cola based drinks (Lussi et al. 2012; O'Sullivan & Curzon 2000; Lussi & Carvalho 2015). First barriers from plaque, salivary pellicle over the enamel crystals must be

breached before damage to the tooth mineral can occur. Hydrogen ions are dissociated from the acids when dissolved in water. These ions interact with hydroxyapatite crystals causing dissolution by either combining with the carbonate or phosphate ion present in the crystal releasing all the ions from that region of the crystal and leaving the typical honeycomb etched surface as shown in the SEM image in Figure 3 (Featherstone & Lussi 2006).

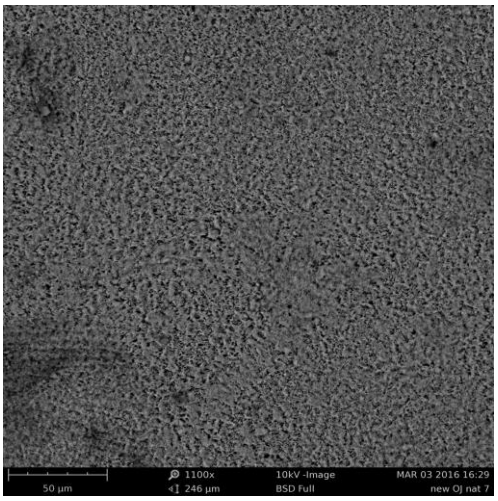


Figure 3: SEM image demonstrating the typical honeycombed appearance of eroded enamel where the core of the enamel prisms has been dissolved by acid, and the adjacent interprismatic areas appearing more pronounced creating a typical appearance of type 1 enamel.

The different acids have different mechanisms of causing erosion. Hydrochloric acid dissociates completely in water releasing hydrogen ions and chloride ions. The hydrogen ions directly dissolve the mineral surface, but there is no effect from the chloride ions; whereas citric acid has a more complex interaction. In water, it exists as a combination of hydrogen ions, acid anions and acid molecules (the ratio of each determined by the acid dissociation constant and the pH). The hydrogen ion behaves exactly as previously described and the citrate anion may interact with calcium removing it from the crystal surface. Therefore, it could be said that citric acid doubly attacks the tooth surface. Citric acid is dominant in the diets of those diagnosed with dietary erosive tooth wear and therefore is an ideal

erosive solution for investigations regarding dietary erosion. These can be either used in pure acid form or using a citric acid based substrate such as orange juice (Austin et al. 2011; Mullan et al. 2017b).

In vitro studies have the benefit of being able to either use commercial products or pure acids. Studies which aim to investigate intrinsic acid use hydrochloric acid to mimic the acidic conditions produced by vomiting (Mann et al. 2014), particularly as gastric juice is difficult to source. However, studies investigating erosive tooth wear from dietary origins may use pure citric acid or citric acid based products (O'Toole et al. 2015; Mistry et al. 2015; Austin et al. 2015; Hooper et al. 2007; Ren et al. 2009). Austin et al. (2016) immersed enamel samples in 0.3 % citric acid with an adjusted pH of 3.2 (50 mL per sample) for 30, 60, 120 and 300 seconds recording microhardness and surface roughness measurements after each erosion time. Whereas, O'Toole et al. (2015) and Mistry et al. (2015) immersed samples in citric acid using same concentration and pH but 8 mL per samples and using a cycling approach. Immersing the samples for 10 minutes then rinsing with distilled water and completing a total of five cycles. Microhardness change and tissue loss measurements were only carried out after completion of all five cycles. Hooper et al. (2007) conducted a combined *in situ* and *in vitro* study to investigate the influence of toothpaste for protection against erosion. For their *in vitro* erosion samples were immersed in citric acid based orange juice for 20 minutes. Ren et al. (2009) immersed samples in orange juice for 5 x 20 minute cycles to replicate a 5-day daily exposure of dietary acid. Using pure citric acid provides the operator with a more controlled and standardised substrate. However, since erosion studies are intended to simulate a real life phenomena the use of commercial products provide a more realistic approach. Furthermore, there is interest in investigating sports drinks (Milosevic 1997; Hooper et al. 2004; Venables et al. 2005; von Fraunhofer & Rogers 2005; Hooper et al. 2005; Rees et al. 2005; Kitchens & Owens 2007; Ostrowska et al. 2016; Melo et al. 2016). Sports drinks have been shown to have erosive potential from their pH, titratability and buffering capacity (Milosevic 1997). This increase in interest in sports drinks may be related to the marketing power of these products and the recent publicity with regards to the oral health of elite athletes (Ashley et al. 2015). Melo et al. (2016) investigated three carbohydrate-electrolyte sports drinks which

are specifically marketed to enhance sporting performance. Investigation of the erosive potential of products and the effect of immersion of enamel samples in the products on microhardness change and tissue loss. All three products had significant erosive potential resulting in enamel softening and tissue loss.

The erosive potential of acidic beverages is related to the pH and the buffering capacity of the product. The more a product resists pH changes from salivary buffering the more it increases the duration of acidity in the oral environment. Edwards et al. (1999) compared the pH and buffering capacity of fruit based and non-fruit based acidic beverages (carbonated and non-carbonated). There were significant differences between the fruit based and non-fruit based beverages suggesting that acids derived from the fruit influence the buffering capacity. The study also identified that initial pH was not indicative of erosive potential, therefore buffering capacity and titratable acidity are better outcome measures of erosive potential. Lussi et al. (2012) also stressed the importance of buffering capacity in a study which investigated the erosive potential and the chemical properties of 60 different agents including beverages and medications. They identified a strong relationship between pH and buffering capacity of drinks and their erosive potential. The study also suggested that the presence of fluoride, calcium and phosphate can reduce erosive potential. This supports previous work where a research team developed a blackcurrant beverage modified with calcium and phosphate over a series of studies. They identified that the addition of calcium to a citric acid based beverage reduced the erosive potential. They compared the erosive effects of their modified beverages versus standard low pH fruit based beverages, consistently showing reduced erosive effects for the modified beverages (Hughes et al. 1999a; West et al. 1999; Hughes et al. 1999b).

As well as modifications to acid based drinks affecting erosive potential, different acids will naturally have different erosive potential. Meurman and Frank (1991) compared the effects of immersing bovine enamel samples in either a cola based phosphoric acid containing beverage with citric acid and malic acid containing sports drinks. The samples immersed in malic acid took longer to exhibit

structural breakdowns compared to those immersed in citric and phosphoric acid containing beverages. The samples immersed in citric and phosphoric acid containing beverage exhibited identifiable changes after 15 minutes immersion, whereas the samples immersed in malic acid containing beverage only showed changes after 30 minutes this is an example of the double erosive effect citric acid can produce, which was mentioned previously. After 30 minutes erosion, there was no statistical difference amongst any of the groups regardless of which acid containing beverage was used suggesting saturation. West et al. (2000) compared tissue loss of human enamel and dentine samples following immersion in citric acid, lactic acid, malic acid at and phosphoric acid. They used a three-cycle erosion regime with an immersion time of 10 minutes for each cycle with measurements recorded at each cycle. They identified that phosphoric acid resulted in significantly more tissue loss in dentine compared to the organic acids however, there was no difference for enamel. Whilst malic acid resulted in the least tissue loss amongst the organic acids it was not significant. Therefore, citric acid should be used for studies investigating dietary erosion as it is the most common dietary acid. Schlueter et al. (2016) compared the erosive effect of three different acid compositions namely native citric acid (unbuffered) (1, 0.5 and 0.3 % citric acid at pH 2.3, 2.5 and 2.8, respectively) and 0.3 % citric acid buffered to pH 3.6. They identified a non-linear effect between decreased pH and increased tissue loss suggesting that titratable acidity as well as pH of acid is important. They identified that erosion with 1 and 0.5 % citric acid produced distinct tissue loss, but not at a concentration of 0.3 %, regardless of the pH. However, the concentration commonly found in natural and commercial products is approximately 0.3 % therefore it provides a more clinically relevant substrate (Hughes et al. 2000)

After selecting the appropriate type of acid/ acid based product to investigate decisions regarding immersion time, temperature and agitation must be made. Erosion studies aim to investigate different phases of the erosive process and different methods to measure erosive changes require different times for changes to be identifiable, for example, for tissue loss to be measurable it requires greater erosion time than for microhardness change. Young et al. (2011) suggested a balance between selecting an immersion time suitable for the methods being used to assess the samples and being

clinically representative is needed, emphasising that the pH only remains low for 2 minutes clinically. Despite this short duration of a low pH irreversible hard tissue loss occurs clinically which is likely to be from the cumulative effect of acid exposures. Consumption of acidic drinks and foods are not completed in one sitting for example a 330 mL beverage may be sipped slowly over a prolonged time period. In a questionnaire where participants were asked the duration a single acidic beverage was consumed; either less than 5 minutes, 5 to 10 minutes or longer than 10 minutes the majority reported the time being more than 10 minutes (O'Toole and Bartlett 2017). Therefore, the time taken by individuals clinically to consume acidic beverages is variable, as is the number of acidic beverages per day. This is reflected by variation of immersion times in the literature. Increased frequency of acidic consumption results in an increased exposure time to a low pH (Schlueter and Tveit 2014). Kitasako et al. (2017) identified a correlation between increased frequency of acidic drinks and prevalence of erosive tooth wear amongst adults. For *in vitro* studies repeated cycling is often used to simulate the cumulative effect of dietary intake which occurs clinically.

Finke et al. (2000) measured enamel loss of natural unpolished enamel over increasing immersion times. Samples were immersed in either lemon and lime juice, a 'tooth kind' blackcurrant juice or water for 15 minutes, 30 minutes, 1 hour, 2 hours and 3 hours with measurements recorded at each interval. To quantify enamel loss the step height difference was measured between a reference area, which had been coated in gold to protect it during the immersion, and the eroded area. The authors refer to a linear trend of increased enamel loss and increased immersion time. Whilst this was evident when lemon and lime juice was used as the erosive substance, the relationship with the commercially available blackcurrant juice was more complex. Statistically significant changes in enamel height was only identified after two hours' immersion in the blackcurrant juice and the results for the lower immersion times were similar to those for water (the positive control). Gonçalves et al. (2012) investigated erosive effect over increasing immersion times. Polished bovine enamel samples were immersed in grape juice (4 juices were compared) for 10 minutes four times a day for a total of 15 days using fresh solution for each immersion. After day five all the groups demonstrated significantly

decreased microhardness with one group showing statistically more hardness change than the others, suggesting increased erosive potential. However, by day 15 all groups had become significantly softer but there was no difference between the groups suggesting a plateau effect. Surface roughness increased following erosion but there was not a consistent linear effect for any of the groups. However, enamel loss calculated from calcium loss had a linear relationship with increased erosion time (Jager et al. 2012). Overall when using the same acidic substrate investigating different or increasing erosion times can be extremely useful. However, when comparing different beverages it can create a more complex situation as the erosive potential of different beverages can also vary at different rates. Mann et al. (2014) measured Sa roughness of polished enamel at baseline and after 30 seconds, 60 second and 120 seconds' immersion in hydrochloric acid. They reported early significant increase in Sa roughness which plateaued after 60 seconds. This shows the importance of comparing different immersion times as initial changes that are identified in a study may not be directly comparable to a progressive effect, and erosive tooth wear is a progressive disease.

Further considerations for an erosion model include temperature, agitation and flow rate. It is accepted that temperature influences chemical reactions. For erosion studies room temperature is obviously most convenient however, soft beverages are often consumed chilled. The temperature in the mouth equates to body temperate 37°C and introduction of hot or cold beverages will result in changes. West et al. (2011) suggested that temperature in studies should be cooled and controlled as increased temperature increases erosion. Amaechi et al. (1999) compared mineral loss and erosion depth following immersion in orange juice at 4°C, room temperature (20°C) and 37°C identifying significant increases in mineral loss and lesion depth with the increase in temperature. West et al. (2000) investigated the effects of increasing the temperature of 0.3 % citric acid on tissue loss of enamel and dentine, identifying a correlation between increased temperature and increased tissue loss. Furthermore, Barbour et al. (2006) compared hardness change and tissue loss following immersion in acidic beverages at 4°C, 25°C, 50°C and 75°C identifying significantly increased hardness change and tissue loss with increased temperature. However, depending upon resources available it

may not always be possible to refrigerate products and a sensible approach would be to ensure temperature stability in preference to short term refrigeration. Where commercial products are concerned, storage should be in accordance with manufacturers' instructions.

In a study which investigated the influence of temperature and flow rate on erosive potential Eisenburger and Addy (2003) identified that tissue loss increased with increased temperatures and increased agitation. To compare temperatures enamel samples were immersed in citric acid at 4°C, 20°C, 35°C or 50°C all under agitation at 270 rpm using a rotary stirrer. To investigate flow rates temperature was kept constant for all groups at 35°C, agitation using the rotary stirrer was doubled and static immersion was investigated. They also investigated the influence of slow laminar flow of citric acid versus jet flow to simulate drinking through a straw. They identified increases in tissue loss with increase in liquid temperature and with increased agitation. However, increased enamel softening did not correlate with increased agitation. Shellis et al. (2005) also identified that velocity or flow rate influenced erosive potential as well as temperature and duration. Their results correspond with clinical links between behavioural habits such as swishing and inappropriate use of straws influencing the pattern of erosive tooth wear. It is unlikely for *in vivo* erosive challenges to be static therefore studies often use agitation during erosion. Furthermore, Mistry et al. (2015) investigated the effects of three types of agitation machines (which use different shaking mechanisms orbital, Gyro and See-Saw) at four different speed settings 30, 40, 60, and 70 rpm. Both the type of agitation machine and the speed settings influenced the results, but generally speaking increasing the speed of agitation increased tissue loss. Schlueter et al. (2016) also compared agitation methods between eroding samples with either a smooth (immersion in a water bath) or a jerky motion (shaking plate) with significant increases in tooth loss for immersion with jerky motion. Therefore, when considering erosion models the type of acid, duration, volume temperature and agitation methods must all be considered and standardised where possible. Using acids at room temperature allows for continuity and the choice of agitation should be maintained throughout.

1.2.4 Remineralisation

Investigations into the remineralisation of enamel following erosion are important for understanding the nature of the disease and possible prevention and treatment. Fluoride is advocated for the remineralising of enamel following demineralisation caused by caries and/or erosion. Fluoride can substitute for hydroxyl ions in the hydroxyapatite crystallites which have become dissociated during the erosion process, forming a partially fluoridated hydroxyapatite Ca1O(PO4)6(OHxFy) (Ten Cate & Featherstone 1991). Natural saliva has been identified as having a comparable remineralising effect to fluoride *in vitro* (Amaechi & Higham 2001b). Eroded bovine enamel samples were immersed in either artificial saliva, natural saliva (or a fluoride solution for 28 days using mineral analysis and lesion depth as the outcome measures. All three models identified significant reductions in lesion depth and mineral loss compared to the control (pre-eroded) samples which had not been immersed in any solutions. However, there was significantly more mineral loss when samples were immersed in artificial saliva compared with natural saliva and fluoride. Natural saliva produces a remineralising effect from the saturated content of calcium and phosphate ions and possibly proteins, however whilst it contains some fluoride ions these are thought to have a minimal effect, and artificial saliva remineralises via a similar process (Amaechi & Higham 2001a). Although there may be a synergistic effect when combining the effects of these individual agents (fluoride, calcium and phosphate). This has been investigated in products with regards to dental caries but it remains an area which requires further investigation in erosion studies (Arafa 2017).

However, *in situ* studies can combine the effects of topical remineralising agents such as fluoride with natural remineralising from saliva. Maggio et al. (2010) compared the remineralising and anti-erosive effect of combining toothpastes and mouth rinses *in situ*. They identified significant microhardness recovery and erosion protection with increased fluoride particularly when toothpastes and fluoride rinses were used in combination. Creeth et al. (2015) investigated the dose-response effect of fluoride toothpastes on remineralisation *in situ*. They compared the effects of paste containing different

concentrations of fluoride and identified a linear correlation with increased fluoride concentration and increased remineralisation and erosion resistance.

Eisenburger et al. (2001a) investigated the effect of increasing immersion times on the remineralisation of enamel. Human enamel samples were eroded in citric acid for 2 hours then immersed in artificial saliva to remineralise for 1, 2, 4, 6, 9 or 24 hours. Profilometry was used to measure lesion depth and SEM was used qualitatively. SEM identified the presence of mineral deposits for each remineralisation exposure and profilometry identified decreased lesion depth with increased immersion in artificial saliva. They suggested that softened enamel had the potential to remineralise if it remained undisturbed. Therefore, tooth brushing is not advocated directly following an acid attack. They also suggested a remineralisation plateau after 6 hours. This has similarities to previously discussed plateaus in regard to surface changes occurring during increasing erosion immersion.

Austin et al. (2011) investigated the effect of applying fluoride varnish as a preventative measure prior to erosion and erosion-abrasion cycling. They identified little protective effect after nine-cycles. There was an initial therapeutic effect with only one product following six-cycles of erosion. This study was inconclusive regarding therapeutic effect of topical fluoride, however the study used a harsh erosion regime to investigate one single application of fluoride. The cumulative effect of continued fluoride application, which is more representative of the clinical situation, was not explored.

Oral health advice given to patients advises them to avoid tooth brushing directly after an erosive challenge instead using a neutral or remineralising alternative (Amaechi & Higham 2005). This begs the question are fluoride based products more effective before, after or between acid attacks? O'Toole et al. (2015) investigated the timing of single dose fluoride application, whether it was more effective before an acid attack or after. They reported that stannous fluoride produced significantly lower step heights when applied before acid immersion compared to sodium fluoride, at similar concentrations, which was more effective after erosion and is better for remineralisation. This was

followed by a subsequent study investigating the therapeutic effects of stannous and sodium fluoride after single dose erosion compared with erosion cycling (O'Toole et al. 2016). For the erosion cycling, the fluoride was also applied during each cycle. They identified significant reductions in step height measurements from both fluorides after a single erosion, however when erosion cycling was considered only stannous fluoride had a significant effect. The authors reported that this may be linked to the cumulative effect of stannous fluoride during the erosion cycling. For the single erosion cycle the fluoride was only applied before erosion. The authors had previously identified that sodium fluoride was more efficient when applied after erosion, so it is interesting that the cumulative effect in the five cycles was not more pronounced. Subsequent studies have concurred with these findings that stannous fluoride may provide better anti-erosion properties than sodium fluoride with enamel samples treated with stannous fluoride toothpaste demonstrating significantly less tissue loss than those treated with sodium fluoride (West et al. 2017). Therefore, the type of fluoride, the concentration of fluoride, the mode of fluoride delivery, the timing of application and the duration of immersion all need to be considered when developing an effective anti-erosion model.

1.2.5 Metrology Terminology

There is specialised terminology in metrology which is specific and distinct from the everyday uses. There is often reference to 3D and 2D roughness analysis, however this may be misleading and it has been suggested the term 2½ D is actually more appropriate (Leach 2014). The difference between 2D and 3D parameters is that 2D parameters such as Ra are calculated from Z heights from a single profile line whereas 3D parameters (e.g Sa) are calculated from all the profile lines in a scanned surface. Whilst the principle of 2½ D may be appropriate, for simplicity and to reduce confusion this thesis refers to the terms 2D and 3D. Resolution is another term with importance in metrology, it is the smallest increment a measurement system can move. It is governed by the size of the stylus or light source and the type of surface measured (Durakbasa et al. 2011). The resolution of an instrument governs its measurement capabilities and previous work suggested that a minimum resolution of 2.5

µm was required to successfully characterise surface texture of human enamel (Austin et al. 2015). This thesis challenges that estimation.

1.2.6 Traceability of measurement systems

In metrology traceability is the basis for which measurements can be considered accurate (Leach 2014). Traceability is defined as "*property of a measurement result relating the result to a stated metrological reference through an unbroken chain of calibrations of a measuring system or comparisons, each contributing to the stated measurement uncertainty*" (ISO 2004). Therefore, where measurement equipment is not traceable it is important to carry out investigations to understand the measurement capability, particularly for substances, which are not defined by ISO standards, such as enamel.

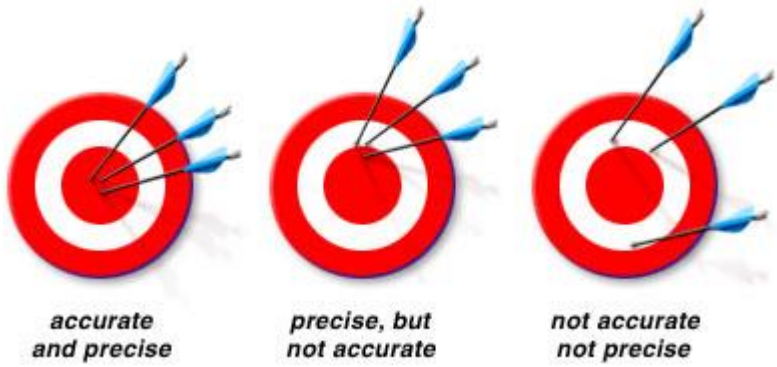


Figure 4: Illustration explaining the differences between accuracy and precision in metrology.

Accuracy is the closeness in agreement to a true known value, whilst precision is the closeness in agreement to a series of measurements and is combined from repeatability and reproducibility (Leach 2014). Repeatability is defined as the closeness of the agreement between the results of successive measurements carried out under the exact same conditions and reproducibility is the closeness of agreement of a series of measurements under changed conditions (Leach 2014). These are shown in Figure 4. The origins of repeatability and reproducibility studies are in engineering and are used for

the validation of measurement equipment and analysis tools (Stanley & Rasberry 1998). The repeatability of a measurement provides the information on the precision of the instrument's measuring capability. Understanding the measuring capability of the equipment enables the user to better understand the value of the results: the resolution and noise of an instrument can help when interpreting results and are essential to instrument calibration. As previously mentioned the resolution is the smallest detectable movement of the instrument and is influenced by the diameter of the stylus or light source used to measure as well as the type of structure being measured (Giusca & Leach 2013; Hocken et al. 2005; Durakbasa et al. 2011). The inherent error (noise floor) is a combination of the instrument's internal noise (instability in the instrument electronics); environmental noise (temperature, floor vibrations) and the noise of the x and y drive units in the measurement along the z-axis when scanning.

Repeatability and reproducibility are terms commonly used in erosion studies. Rodriguez et al. (2012a) conducted a repeatability and accuracy study to assess the precision and accuracy of a no-contacting profilometer and surface mapping software, firstly using a steel block of known dimensions to determine the accuracy of the instrument and software and then carrying out measurements of dental casts representing tooth wear changes. Louwerse et al. (2004) carried out a study to investigate the reproducibility of ultrasonic enamel thickness measurements. They assessed the ability of different operators at selecting the same regions of enamel, which had surface fiducial markers. They compared the results of four operators who each marked 12 teeth at baseline and repeated the process after one week. Due to errors in precision, they suggested reliable measurements could only be achieved with a minimum lesion depth of 0.33 mm. Mullan et al. (2017b) investigated the measurement uncertainty of a white light profilometer suggesting the standard combined uncertainty was ±0.28 µm. The main source of the measurement uncertainty was from flatness errors relating to the x-y movement of the stage. Within the field of dentistry, many varying studies have adopted the use of repeatability and reproducibility techniques to validate their results. Studies to assess the difference of dental materials often use these techniques to compare products. Shah et al. (2004) compared two

impression materials to replicate a stone cast of a human dental arch. The repeated impressions and the stone cast were scanned and analysed using a 3D laser scanner with supporting analysis software (Shah et al. 2004). Recently reproducibility techniques have been adopted to investigate electronic colour measurement guides, which could revolutionise aesthetic dentistry (Weyhrauch et al. 2015).

1.2.7 Profilometry

Contact profilometers (CP), non-contact profilometers (NCP) and confocal laser microscopes (CLM) can be used to measure surface roughness and step height, further details of which are described below. Contact profilometry works by a metal stylus moving and tracing over the surface of the specimen and the change in amplitude is recorded with any displacement but it only provides 2D information and risks damaging the measured surface (Macdonald et al. 2010; Field et al. 2010). The two main types of CP are stylus profilometers and scanning probe microscopes. Stylus profilers move a probe across a surface (either the arm of the probe moves or it remains static and the sample is moved) vertical displacement of the stylus is detected by a linear variable differential transformer (LVDT) and this signal is converted to amplitude data (Schmit et al. 2007). Scanning probe microscopes are also referred to as Atomic Force Microscopes and have higher resolution. Chappard (2003) claimed that CP are limited to flat surfaces and recommended 3D image analysis for soft or complex surfaces as measurements are limited by the size and shape of the stylus tip as shown in Figure 5.

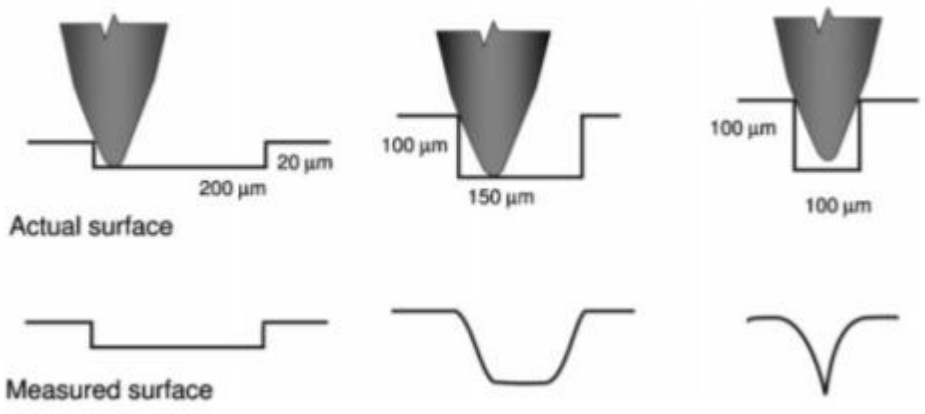


Figure 5: Schematic demonstrating the limitations of a 25 µm stylus (Schmit et al. 2007)

NCP and CLM use a light source in place of the stylus to scan the surface. The principal of confocal is applied where light is reflected back from the surface and the displacement used to build up 3D representation of the surface line by line. Confocal differs from standard microscopy with the use of apertures that ensure only light at the point of focus on the measured surface enters the detector eliminating out-of-focus and stray light, therefore producing higher resolution images as demonstrated in Figure 6 (Schmit et al. 2007; Hocken et al. 2005). These systems scan the measured surface in an X-Y raster pattern, either by the light source moving or the measured surface being moved upon a stage and resulting in a representation of the sample at a given focus plane (Schmit et al. 2007). Confocal profilometers and CLM are high resolution systems capable of detecting microscopic changes and ideal for surface topography.

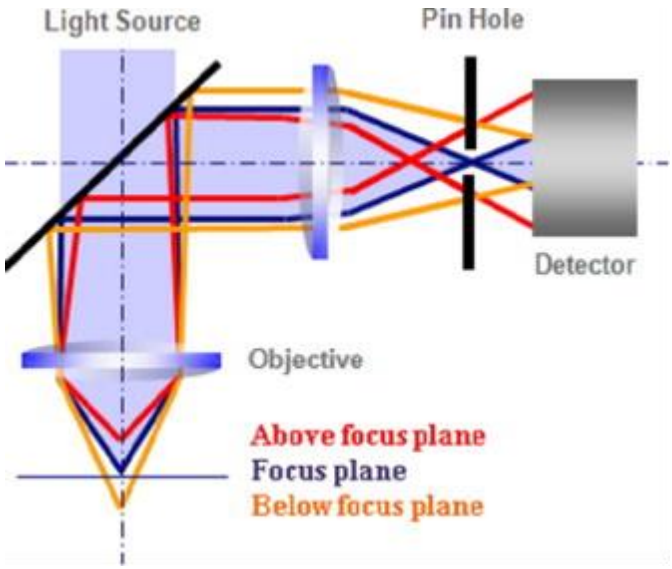


Figure 6: Diagram demonstrating the principal of confocal imaging.

However, similar to contact profilometry, the overall shape and type of the measured surface influences the capability of the system. Specifically, accuracy, resolution and precision all differ for flat and curved surfaces. Hewlett et al. (1992) demonstrated this by measuring a sphere gauge using a

contact profilometer and reported that accuracy and precision decreased over sloped areas. The light source of an optical system distorts and elongates over slopes reducing accuracy and precision (Jovanovski & Lynch E 2000). In a recent study which investigated natural unpolished enamel with signs of erosive tooth wear, Ranjitkar et al. (2016) noted that measuring the sloped regions of natural samples resulted in increased drop out from the laser and subsequently discounted scans with excessive drop out. These observations highlight the increased error when measuring curved surfaces and in these instances smaller areas from regions of optimal focus should be selected i.e. the apex of the curvature. Another consideration is the complexity of the structure of enamel, as discussed in detail earlier prism orientation differs throughout (Braly et al. 2007). Furthermore, outer enamel contains both aprismatic and prismatic enamel resulting in complex surface texture (Whittaker 1982). These, complexities result in natural unpolished enamel being difficult to measure accurately, however the differences between polished and natural unpolished enamel indicate that these measurement difficulties must be overcome to obtain clinically relevant information. This thesis aims to develop a method to quantify the surface texture of natural unpolished enamel by minimising these measurement difficulties.

Paepegaey et al. (2013) compared the ability of a CP, NCP and CLM to identify tooth surface loss following erosion. Six groups of polished enamel samples (8 samples per group) were measured for step height loss by three examiners using the three operating systems. By using the CLM last, they were also able to qualitatively assess the samples, noticing that scratch marks were left behind by the CP. The suggested the depth of these to be less than 0.5 µm as they were unable to measure them at a profile level. However, changes as small as this would affect roughness output measurements. All three instruments were able to reliably detect surface loss and whilst each method produced different depths for the same areas measured there was strong inter method agreement. The CP resulted in the lowest depth figures, which is unsurprising due to the mechanism of the stylus. The highest depth figures were from with the NCP which was a confocal system with a white light source and a 7 µm spot size. The CLM was the highest resolution device and was traceable, and therefore considered the most

accurate and precise of the systems tested. Overall, the study identified that whilst the individual results may not have been comparable from device to device, the overall trends were the same despite the differences in resolution.

1.2.7.1 Surface roughness

A surface is made up of form (profile), waviness and roughness (combined as texture) (DigitalSurf 2013) as shown in Figure 7. The primary profile can be used to determine profile parameters and is used for tissue loss measurements which will be described later (Leach 2014). For roughness to be extracted a selected filter is required to suppress the long and mid length wavelengths relating to profile and waviness. Field et al. (2010) described roughness as deviation from the form of a surface, however, another interpretation could be deviation within the form as it is only after the three components are separated that each can be analysed individually.

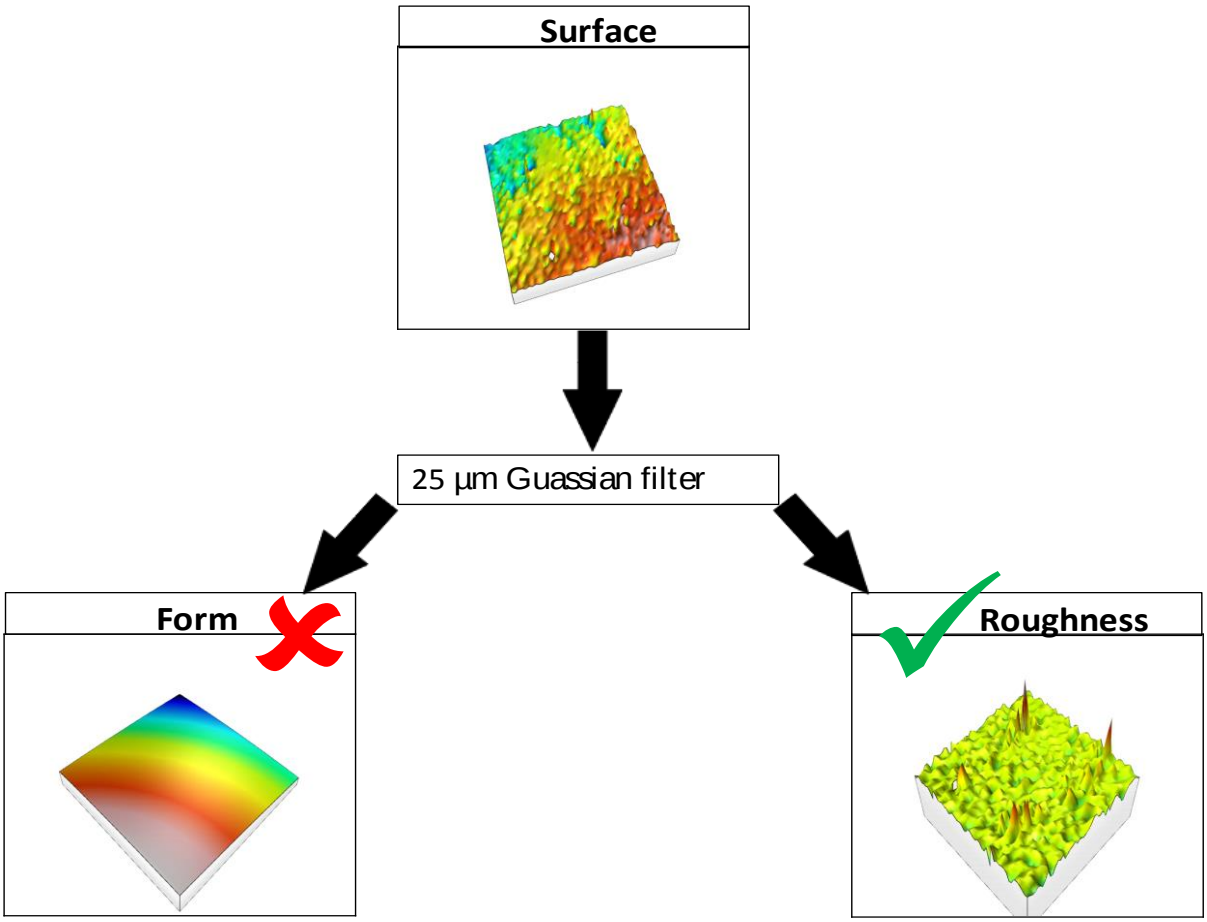


Figure 7: Image demonstrating how an overall 3D surface is combined of form and roughness which can be extracted using filters

There are different parameters which can be used to calculate the roughness of a surface, including height and hybrid parameters. Height (or amplitude) parameters measure the vertical deviations from the form (Gadelmawla et al. 2002). Ra (roughness average) is one of seven height amplitude parameters and is a 2 dimensional measurement, it is the average height deviations from the primary profile of a surface (one line). The mean line for the primary profile is a reference line for parameter calculation and is determined by fitting applying the least squares method which in essence is removing nominal form from the measurement process (Leach 2014; DigitalSurf 2013). Figure 8 illustrates the derivation of Ra. The centre line is identified and the areas of the graph originally below centre line are placed above. Therefore, Ra is the mean height of the resulting profile.

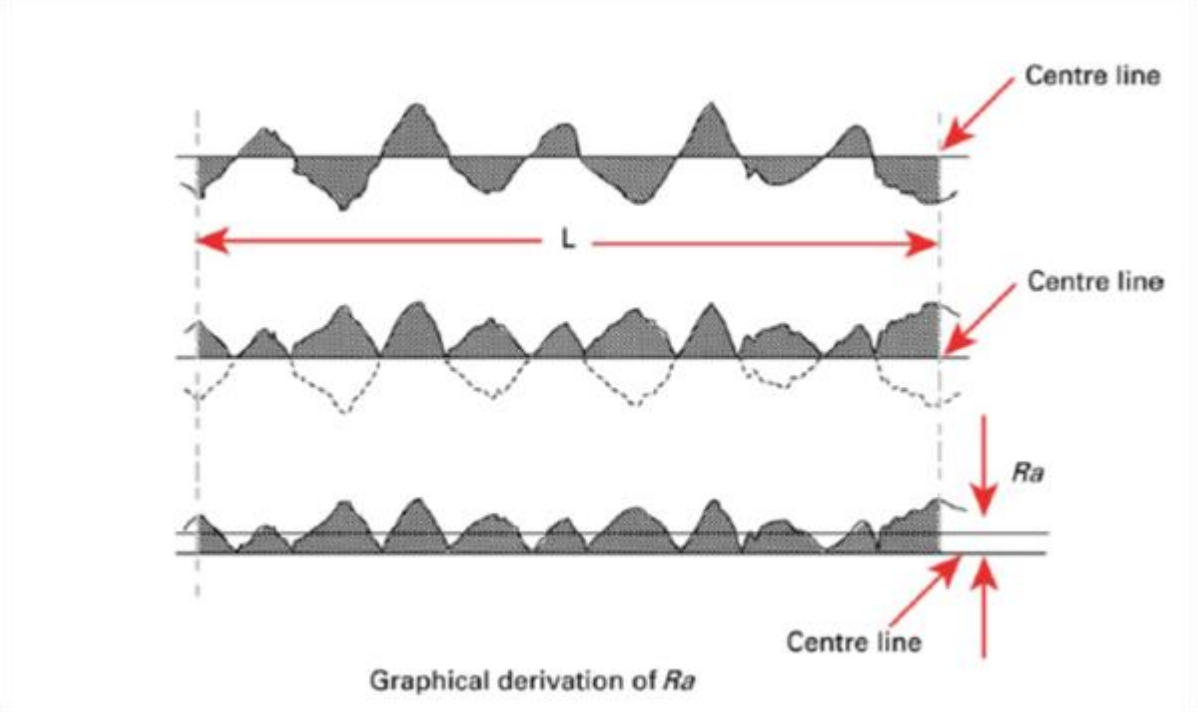


Figure 8: Derivation of Ra roughness (2D arithmetic mean)

Sa is the 3 dimensional equivalent of Ra and is the mean roughness of an overall measured surface, and therefore is more representative than Ra, as shown in Figure 9. Definitions of the 3D parameters are shown in Table 5.

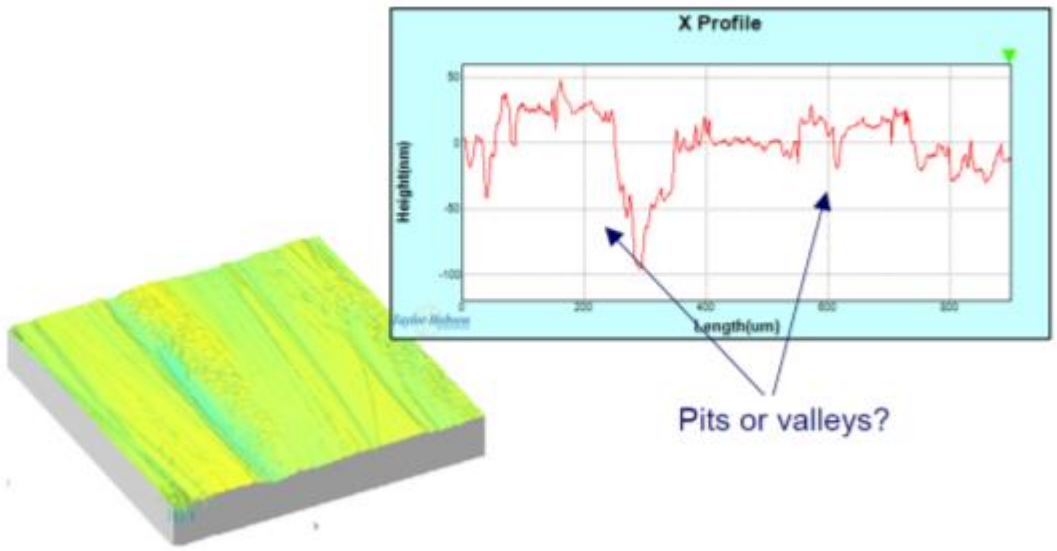


Figure 9: Illustration demonstrating the ambiguity of 2D roughness measurements compared to a 3D representation of the same surface.

Table 5: 3D amplitude parameters table.

Sa	Average mean height
Sq	Root mean square height
Ssk	Skewness
Sku	Kurtosis
Sp	Maximum peak height
Sv	Maximum pit height
Sz	Maximum height
Sdr	Developed interfacial area ratio, which is the percentage of additional surface area contributed by the texture as compared to an ideal plane the size of the measurement region

However, the most commonly used roughness parameter in dental research to quantify surface changes still remains Ra (Field et al. 2010). Willershausen et al. (2008) measured baseline Ra roughness of polished deciduous and permanent enamel samples and following incubation in apple juice after 2, 8 and 24 hours. Roughness measurements were carried out using a non-contact profilometer, calcium analysis and qualitative assessments were also conducted as markers of erosion. Both types of polished enamel increased in Ra roughness following erosion. Soares et al. (2015) investigated the anti-erosive potential of fluoride varnish and gel using Ra roughness, mineral analysis and qualitative high resolution imaging. They measured Ra of polished bovine enamel samples using a contact profilometer with a 4 µm stylus and a 0.25 mm cut off calculating Ra from just 3 profile tracings per sample. They identified that samples which were coated in fluoride protective products

demonstrated lower Ra roughness values post erosion which, combined with the lower level of mineral loss identified in these samples, indicated a positive anti-erosion effect. Derceli et al. (2016) measured Ra roughness of polished bovine enamel samples at baseline and after exposure to HCL for 10, 20, 30 and 40 seconds. They identified significant increase in roughness but noticed that it tended to stabilise after 30 seconds suggesting that roughness change is a good method to investigate early erosion. However, questions remain regarding the continued use of 2D parameters.

Alexandria et al. (2017) used both 2D and 3D surface texture parameters to quantify surface roughness, analysing for both Ra and Sa. Interestingly their baseline values for the same samples were very different depending upon the parameter used (Ra = 0.17 ± 0.017 µm and Sa = 0.40 ± 0.04 µm). In general, there is consensus amongst dimensional metrologists that as Sa is calculated from the overall surface it is the more appropriate and representative parameter to use. Understandably there is a trend toward dental studies using the more representative 3D Sa to quantify enamel changes after erosion (Austin, et al. 2015). Ren et al. (2009) investigated changes in surface roughness of polished enamel samples after exposure to orange juice, whitening products and distilled water. They used three roughness parameters Sa, Sz and Sdr. Sa roughness changes indicated a significant increase in roughness when the enamel samples were eroded with orange juice, but no significant changes for the other groups this was reflected in the data for Sz and Sdr. Gracia et al. (2010b) investigated the effect of an anti-erosion treatment by pre-coating samples with the hydrosoluble combination polymer based product with or without added fluoride and comparing the response to erosion of untreated enamel samples measuring Sa and bulk tissue loss. They reported that samples coated with the anti-erosion product had significantly lower increases in Sa roughness compared to the control suggesting a beneficiary anti-erosion effect. Moazzez et al. (2014) used Sa roughness to discriminate eroded enamel samples with or without protection from saliva from either healthy participants or participants with erosion. The samples having undergone immersion in citric acid for 10 minutes were imaged using a white light profilometer and Sa roughness extracted. They reported significant differences in roughness values for the samples without pellicle formation from healthy volunteers,

suggesting a protective effect from the pellicle in participants without erosion. However, this study was slightly limited in that it did not record baseline values for the groups, and therefore the possibility of intergroup differences at this stage cannot be completely ruled out. Mann et al. (2014) measured Sa roughness changes of polished enamel to investigate early erosion induced by gastric acid. They measured Sa roughness at baseline and after 30 seconds, 60 seconds and 120 seconds' immersion in HCL of either pH 1.5 or 3.0. They imaged five areas spread out across each sample (each area was 43 µm by 43 µm). To extract Sa roughness the images were levelled to remove any tilt and an 8 µm Gaussian filter applied. They identified significant increases in surface roughness after only 30 seconds for both pH values, however they suggested a plateau effect occurred after the initial demineralisation. This supports suggestions that roughness change is better at identifying early changes in erosion as opposed to more extensive erosion. The authors selected five areas per sample, one in the centre and four in the periphery the reader can deduce that this was to be representative of the overall sample. However, this was not validated and the areas measured were extremely small 43 by 43 µm with 300 µm left between each area measured only a limited proportion of the sample was examined. Therefore, further work to characterise surface texture of overall area of enamel samples is required.

There are other methods used in dental research to quantify surface roughness, including the bearing curve and area scale analysis (Field et al. 2013; Austin et al. 2015; Las Casas et al. 2008; Arnold et al. 2015). The bearing curve is a hybrid parameter which combines amplitude and spacing as shown in Figure 10. The bearing line is calculated at different heights of the profile (the sum of the samples lengths at that particular height) and plotted to produce the bearing curve which can be used to compare profiles qualitatively and quantitatively (Gadelmawla et al. 2002; Leach 2014; Field et al. 2010). Field et al. (2013) criticised the use of Ra in dental studies as being unrepresentative of the overall surface characteristics and investigated the use of the bearing curve by comparing roughness values between bovine and human polished enamel samples at baselines and after erosion. They used a contact profilometer with a stylus radius of 5 µm. At baseline Ra measurements were unable to

discriminate between the two types of enamel, however bearing parameters MR1 and MR2 identified statistically significant differences between bovine and human enamel ($p<0.001$). After erosion Ra roughness of both enamel types significantly increased but there was no statistical difference between the two enamel types. Whereas, MR1 and MR2 (from the bearing curve) demonstrated statistically significant increases in roughness values for both enamel types and statistically significant differences between roughness values of bovine and human enamel ($p<0.001$). This suggests that Ra was unable to fully represent the overall topography of the samples. However, this would also have been influenced by the limitation of Ra being extracted from a single profile not the overall surface (unlike Sa) and by the limited resolution of the stylus contact profilometer.

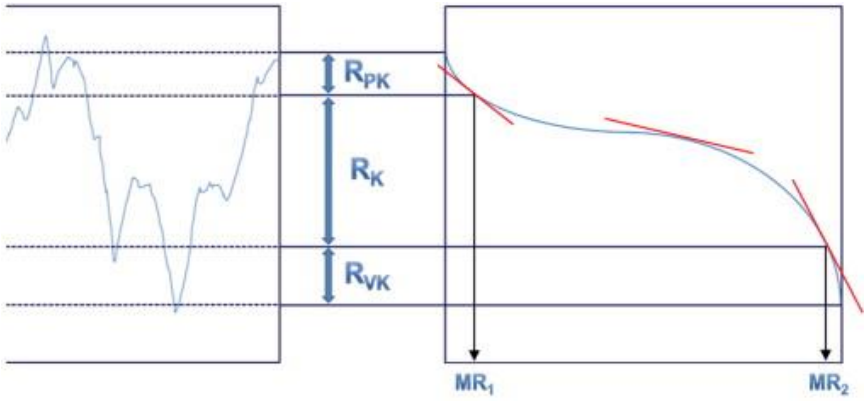


Figure 10: The bearing curve that results as a cumulative distribution of plateaux lengths at the peaks and troughs with MR_1 relating to peaks and MR_2 troughs (Field et al. 2013).

Area-scale fractal complexity (Asfc) is another hybrid parameter, not calculated from amplitude alone, but a combination of amplitude and spacing. It originates from fractal geometry developed by Mandelbrot and Wheeler (1983), where the surface is characterized by its fractal dimension (Brown et al. 1998). Area-scale fractal analysis is based on the principle that the area of a rough surface is not unique and depends on a scale of measurement and it estimates the area of a rough substance as a function of a scale (Brown et al. 1998). The measured surface is analysed using a virtual tiling technique to calculate the relative area based upon the size of the scale used (the size of the individual tile), the

relative area is plotted in a log-log plot against the scale to determine the fractal dimension which is used to quantify the complexity of the measured surface over a variety of scales (Asfc) (Leach 2014; Hyde et al. 2014; Siegmann & Brown 1997, Brown & Siegmann 2001). Higher complexity values indicate rougher surfaces (Ungar et al. 2012). The objective is to characterise the measured surface textures in order that they can be differentiated and correlations established, it is a technique often used to associate wear patterns with different diets (ASME 2002, Ungar et al. 2012). Table 6 and Figure 11 demonstrate analysis and output options provided for Asfc from surface metrology software (MountainsMap DigitalSurf, France).

Table 6: Example of Fractal analysis and output options (DigitalSurf 2013).

	Enclosing Boxes	The method consists of enclosing each section of a profile by a box of width ε (in points) and calculating the area Aε of all the boxes enclosing the whole profile. This procedure is iterated with boxes of different widths to build a graph ln(Aε) / ln(ε). This method can be extended to build a volume graph ln(Vε) / ln(ε) for surfaces.
	Enclosing Boxes in real units	This method is similar to the Enclosing Boxes method, but uses boxes with a width in real size (instead of a width in points).
	Morphological Envelopes	The upper and lower envelopes are calculated by morphological opening and closing using a structuring element which is a horizontal line segment of length ε. Next the area Aε enclosed between the elements is calculated. This procedure is iterated with structuring elements of different lengths to build a graph ln(Aε) / ln(ε). This method can be extended to build a volume graph ln(Vε) / ln(ε) for surfaces.
Slope	**Slope of the regression line**	Both of these parameters are calculated for two regression lines, one connecting the points to the left of the graph, the other connecting the points to the right. This makes it possible to analyze multi-fractal curves with two different slopes depending on the scales in the analysis.
R^2	**Correlation coefficient of the regression line**	
Fractal dimension		The fractal dimension is calculated from the slope of that one of the two regression lines that corresponds best (i.e. the one out of the two regression lines whose correlation coefficient is nearer to 1 for a profile and nearer to 2 for a surface).

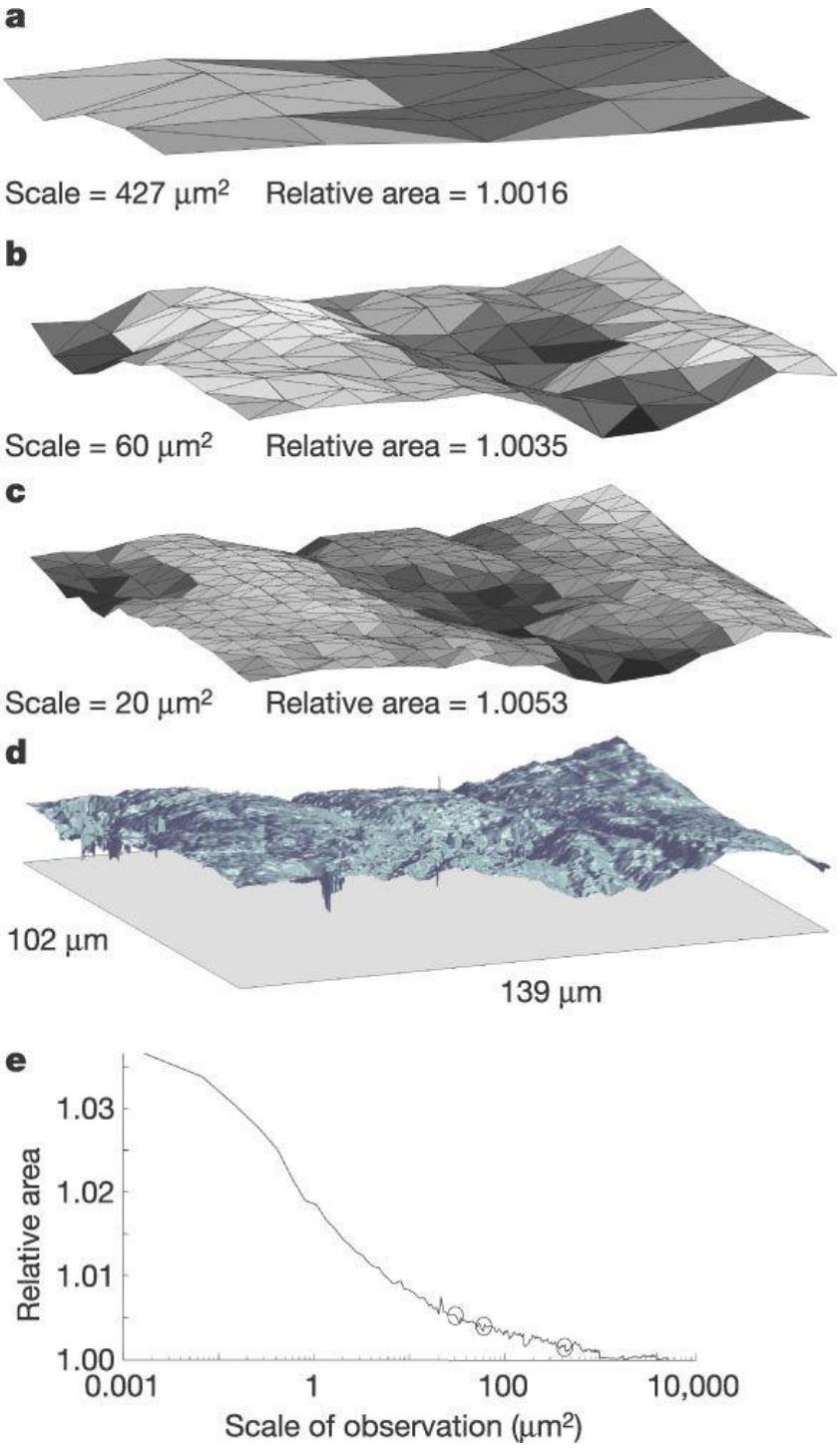


Figure 11: Demonstration of the processes involved in area-fractal scale analysis. The relative area is calculated by dividing the area of a surface (calculated using triangles of a given scale in a virtual tiling algorithm (a, b, c)) by the projected area of the surface (d). Relative area can then be plotted against scale in a log–log plot (e). Asfc30 is a scale-sensitive measure of roughness and is the slope of the steepest part of the curve fitted to the plot of relative area over scale, multiplied by -1,000 (Scott et al. 2005).

Tribology studies which associate diets and wear patterns claim that amplitude parameters such as Sa and Ra are insufficient at providing sensitivity to minor surface changes as they are solely calculated due to height deviations not taking into account wavelengths (Gadelmawla et al. 2002; Sedlaček et al. 2012). However, it is important to remember that tribology and anthropological studies examine mechanical wear abrasion and attrition whereas erosion is the chemical dissolution of mineralised tissue, therefore Sa may be sufficient in this case (Shellis & Addy 2014). Overall there is no doubt that Ra and Sa have been successful in identifying early erosive changes using flattened polished enamel and dentine.

Meireles et al. (2015) claimed that average parameters Sa and Sq were insufficient at distinguishing between worn areas of natural unpolished enamel in a study which compared surface roughness measurements (Sa, Sq, Ssk Sku) of 16 native extracted teeth divided in 8 with wear and 8 without wear. Hara et al. (2016) also had difficulty identifying erosive changes in native natural unpolished compared to polished flattened enamel using average height parameters. However, in a small longitudinal *in vivo* study which used Ra and contact profilometry to quantify surface changes of enamel measuring acrylic replicas of upper anterior segments of 22 participants, Whitehead et al. (1997) noted the surface roughness of enamel significantly decreased over a 3 month period. During the three months participants were exposed to erosive foods and drinks as reflected in their diet diaries which led discussion to a link a link between erosive tooth wear and decrease in surface roughness of natural unpolished enamel. This was an exciting step forward in the approach to measuring erosive tooth wear, however it was not without flaws and therefore could not be considered exhaustive. As mentioned it was a small study with only 22 participants. The authors recorded Ra as an average measurement of the overall specimen. By definition, Ra is only calculated from a single profile and not the overall surface therefore, Ra readings from multiple profile lines would need to be measured, averaged and validated for it to be considered representative of the overall surface. The contact profilometer used in the study had a stylus width of 5 µm, which being the same width of an enamel may be unable to detect changes within this level. Furthermore, whilst

the use of replicas is a natural approach for *in vivo* tooth surface measurement there was no mention made of validation of the materials selected for the study. Therefore, further work to quantify erosive changes *in vivo* is required using 3D parameters to provide accurate representations of the overall surfaces which must begin with measuring natural unpolished enamel *in vitro*.

The methods used to extrapolate surface roughness parameters influence the results. In 3D areal surface texture measurements there are no longer the three distinct groups of 2D profile (Pa), waviness (Wa) and roughness (Ra) and instead appropriate filters are applied to assign the wavelengths to their appropriate group. However, Sa remains Sa irrespective of the filter that has been used, meaning that one has to be aware of the filter applied to understand the wavelengths Sa is representing i.e. waviness or roughness (Leach 2014). Filters will include or reject wavelengths at a pre-defined cut off in order to separate form, waviness and roughness to leave the operator with the desired structure. To determine the correct cut off for roughness there is a rationale which can be used from the waviness and profile wavelengths. A Gaussian filter is a general multipurpose filter that can be used to separate waviness and roughness from a surface in a single process (Leach 2014). The size of cut off for a filter depends upon the type of surface being examined and the wavelengths of the intended structure of the surface, the incident area of the light source or radius of the stylus (spatial resolution) and measurement sampling frequency to be examined for statistical significance (DigitalSurf 2013). Figure 12 expresses a flow chart of the filtering process. The S-filter is a low-pass filter, similar to a waviness filtering with a small cut-off removing unnecessary noise. The L-filter is used to remove unwanted large-scale lateral components of the surface, and the F-operator removes the nominal form. The S filter is defined as the nesting index and set out by ISO standards relating to the width of the stylus and band width to create the cut off. If this is too small the loaded surface can be used on its own as if a filter has been carried out. The filter cut-off is the limit wavelength between waviness and roughness.

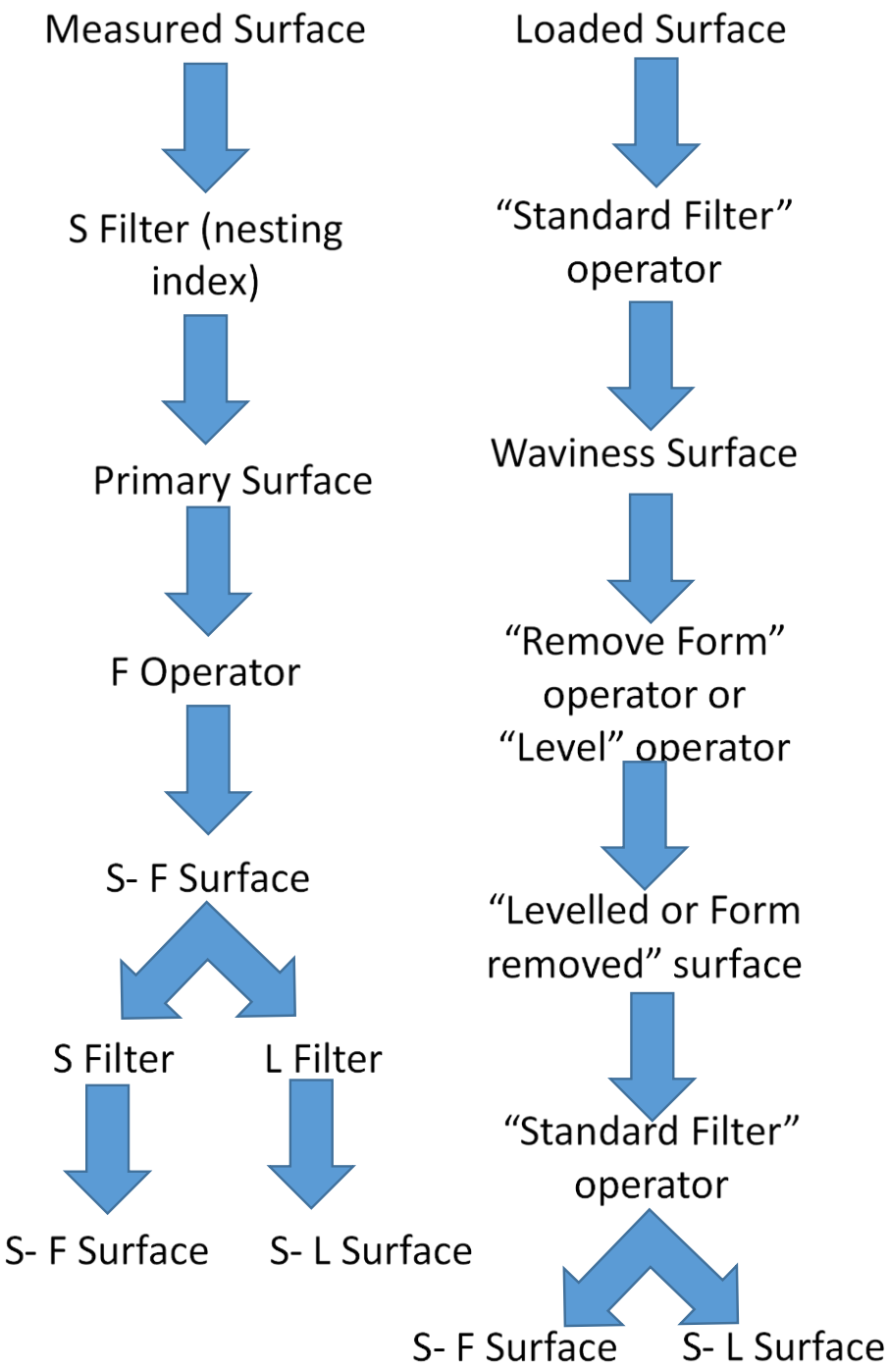


Figure 12: Flow chart demonstrating the filtering process used to extract roughness data. The flow chart on the left hand side describes the functions as defined by ISO standards. The S-filter is a low-pass filter, similar to a waviness filtering with a small cut-off removing unnecessary noise. The L-filter is used to remove unwanted large-scale lateral components of the surface, and the F-operator removes the nominal form. The flow chart on the right hand side describes the functions as defined by the analysis software. Essentially the analysis software follow the protocols set out by ISO standards.

Hara et al. (2016) were unable to identify changes in Sa roughness when using a Gaussian filter of 0.8 mm. An enamel prism is 3 to 6 µm in diameter so to examine within the prism dimension we are investigating roughness at a 1.5 to 3 µm level (Cuy et al. 2002). Therefore, it could be argued that the choice of filter used in the Hara et al. (2016) study was inappropriate as it did not cut off all the waviness wavelengths. It would be recommended to use a smaller filter similar to Austin et al. (2016) who optimised their roughness data by selecting a Gaussian cut off at 30 µm based upon the diameter of an enamel prism. Hara et al. (2016) also measured Asfc, which was able to detect changes following erosion on natural unpolished enamel sample. At first interpretation, this could lead to the conclusion that only Asfc was able to quantify adequate data. However, like choosing a filter for amplitude parameters (Sa). the method of analysis (in this case the size of the scale used) can greatly influence the results. Arnold et al. (2015) used Asfc to identify changes of natural unpolished enamel following erosion with HCL after 2 minutes, 2 x 2 minutes, 3 x 2 minutes and 4 x 2 minutes. They identified significant changes only between 2 minutes and 4 x 2 minutes where the roughness significantly decreased. Ranjitkar et al. (2016) also used Asfc to characterise wear on natural unpolished enamel from erosion and attrition, along with anisotropy. Anisotropy is defined as a difference in a materials' mechanical properties when measured along two axes, it particularly associated with scratches. Their Asfc analysis was iterative measurements based upon the tiling effect described in an earlier paragraph, the scale settings used were 0.02 to 100 µm² at scale 10 and the relative area was created by dividing those totalled areas against the scan area. The logs of the relative area were plotted against logs of the scale used. From the resulting graph, quantitative Asfc values were taken as the value from the steepest part of the graph curvature. They identified that teeth with clinical characteristics of erosion had lower Asfc suggesting that the natural unpolished enamel surface became smoother after erosion. However, this study merely quantified existing surface texture using teeth which had been selected as having existing wear and the authors characterised the aetiology of the wear clinically, albeit with the 2 examiners standardised. This study therefore, cannot truly be determined as solely

measuring erosion or solely measuring attrition as clinical tooth wear is multifactorial in nature. To explore the finding of this study further an *in vitro* study using erosion cycling and laboratory induced attrition would be required, similar to the previously mentioned study by Hara et al. (2016). With regards to deciding upon the scale to use for Asfc, Austin et al. (2016) took a novel approach. In this collaborative study between the National Physics laboratory (NPL) and King's College London Dental Institute the authors combined measurement of surface texture and microhardness changes of polished enamel following erosion exposure in citric acid. To select the optimal scale for Asfc analysis the authors explored the correlation between changes in surface texture at varying relative area-scales to changes in microhardness. This suggested an optimal scale to best highlight the features of enamel was 20 μm^2. However, to apply this to natural unpolished enamel microhardness measurements would have to be conducted on natural unpolished enamel surfaces, which was trialled in Chapter 2 section 2.5 .

Caution must be taken with correlating surface roughness with other measurement techniques. Rakhmatullina et al. (2011) investigated the correlation between surface roughness measurements and reflectometry using polished enamel samples. Their *in vitro* study identified a rise in diffuse and decrease in specular spectrometry along with increases in surface roughness following erosion. As diffuse spectrometry is related to rougher surfaces and specular associated with smooth shiny surfaces there appeared to be a convincing link between surface roughness measurements and reflectometry. This was explored further in a following study in which Rakhmatullina et al. (2013) investigated natural unpolished enamel. They again identified increases in diffuse and decreases in spectral spectrometry, however, surface roughness was not measured but assumed based upon the previous study. Other studies indicate that surface roughness of natural unpolished enamel decreases following erosion (Whitehead et al. 1997; Arnold et al. 2015; Hara et al. 2016; Ranjitkar et al. 2016).

Mullan et al. (2017a) investigated the possibility of a correlation between surface roughness and tubule patency of dentine samples following erosion abrasion regimes. They investigated a tubule

occluding dentifrice against a standard fluoride dentifrice at two brushing forces 100g and 400g. Tandem scanning microscopy (TSM) was used with specialised software to quantify the number of tubules exposed before and after the intervention and surface roughness measurements were also recorded. They identified surface roughness increases for all investigated groups despite the desensitising groups resulting in increased tubule occlusion and the fluoride dentifrice groups resulting in increased tubule patency. Unsurprisingly, there was no correlation between roughness measurements and tubule patency. This study shows the importance of careful interpretation of surface roughness measurement, as two very different occurrences at a profile level may result in similar changes in roughness measurements.

1.2.7.2 Step height

Step height is another measurement technique, which can be carried out with profilometry. A flat surface is preferred for step height measurement and therefore levelling of measured surface is an essential part of the analysis process. This is carried out by algorithms in the analysis software based upon the least square method which is well known in nanometrology (Misumi et al. 2006; Haitjema 1998). The definition of step height from the ISO 5436 standard states that two reference areas are required and a 'step' in the centre as shown in Figure 13.

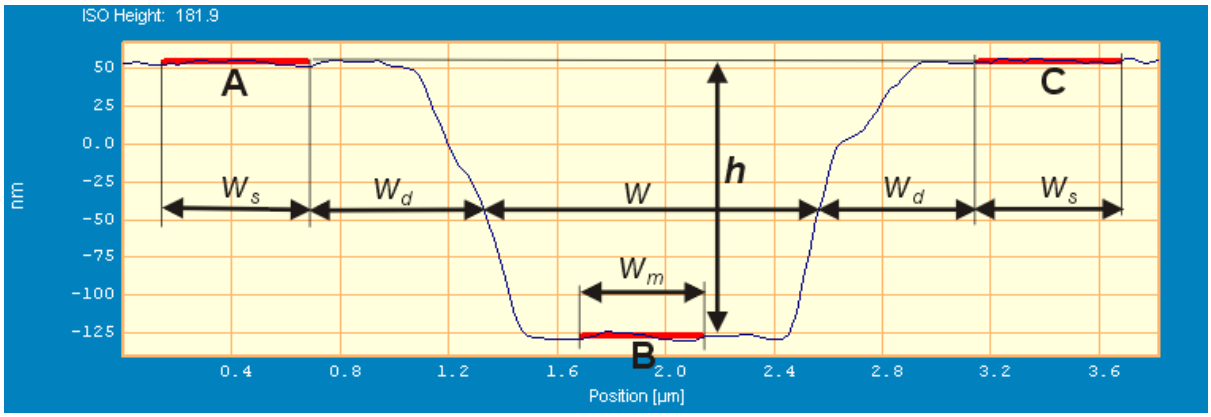


Figure 13: ISO 5436 step height measurement is calculated as the vertical drop from the centre of the trough (B) in relation to A and C on the reference areas and the two measurements averaged.

In the case of erosion studies, the central zone of enamel is exposed to acid whilst the reference areas either side covered by a protective barrier to leave them unaffected by the acid (Mistry et al. 2015). This results in loss of tissue in the central zone recreating the requirements for step height measurements. Ganss et al. (2000) applied an acrylic resin to cover half of each sample, removing the acrylic after erosion cycling with a scalpel. Similarly, Conceição et al. (2015) also only created one reference area of their polished enamel samples, applying nail varnish to half of the sample. However, after erosion there was only one reference side therefore the step height measurements to indicate tissue loss did not follow ISO standards. With only one reference side it would be difficult to judge if the loss in height from the eroded side was a true reflection of the erosive process or an anomaly of the sample itself, particularly for natural unpolished enamel. To overcome this issue baseline profile tracings could have been conducted and used for comparison. A further difficulty when measuring natural unpolished enamel with optical profilometers for step height is the drop out encountered on sloped areas mentioned previously (Ranjitkar et al. 2016).

The two most popular barrier materials used in erosion studies to create reference areas are nail varnish and tape. Nail varnish can be painted on the enamel surface creating a window and removed with acetone, following the erosive challenge (Chan et al. 2014). However, both the application of nail varnish and its subsequent removal with acetone have potential to affect the surface topography of the underlying enamel. Adhesive tape when used is placed over the enamel sample in a similar method to nail varnish (Gracia et al. 2010a). Some authors use alcohol to remove any residue of the tape on the enamel surface (Wang et al. 2014). As alcohol is present in many oral rinses any effects on the enamel topography would be more consistent with *in vivo* conditions. Gracia et al. (2010a) avoided their barrier method affecting their surface topography measurements. After using an acid resistant adhesive tape to create the window with two reference areas for step height measurements, baseline Sa roughness was measured from the exposed window of enamel before erosion and repeated after erosion. Therefore, they optimised their samples for both step height and surface roughness measurements.

It has been suggested that a minimum lesion depth of 0.5 µm is required for optical profilometers to reliably detect changes (Hara & Zero 2008; Schlueter et al. 2011). Hara and Zero (2008) investigated the erosive potential of 10 different acidic beverages. This study was twofold, firstly they characterised the erosive potential of the beverages themselves by quantifying the acidity of each product and secondly, they measured the erosive effects of the beverages namely by measuring step height (tissue loss) and microhardness change. Polished enamel samples were prepared and adhesive tape used to create the window of exposed enamel. Samples (n=10 per group) were divided equally amongst 11 groups (1 group per beverage and 1 control) and immersed in repeated acid cycling for 0 (control), 5, 10, 30, 60, and 120 minutes, tissue loss was measured using an optical profilometer by scanning three experimental windows and four reference surfaces for each sample and calculating the difference in height between the window and reference surfaces. They suggested that profilometry was not sensitive at detecting early changes and a minimum erosion time of 10 minutes would be recommended to identify tissue loss induced by commercial products.

To measure the step height according to ISO 5436 the average height from a selected area from the step zone is deducted from the average height from selected areas from two reference areas (DigitalSurf 2013). However, different analysis software programmes use different mechanisms, some offering multiple choices such as operator-selected single line vertical drop, automatic step height measurement and ISO step height measurement (DigitalSurf 2013). Sancakli (2015) scanned polished enamel samples following erosion only or erosion abrasion regimes with a white light profilometer. The resulting scan images of the samples (with two reference areas and central test area) were analysed for step height using the ISO 5436 function on the surface analysis software (MountainsMap, DigitalSurf, France). This function automatically calculates the step in strict accordance with ISO standards and cannot be influenced by the operator. The authors validated their step height measurement technique to an accuracy of 0.042 µm. Mistry et al. (2015) also used the ISO 5436 technique to measure step height in their study investigating the effect of model variables on *In vitro* erosion studies they used a five-cycle erosion regime resulting in a total erosion time of 50 minutes in

citric acid, thereby providing optimum conditions for measuring lesion depth with an optical profilometer (Mistry et al. 2015). In another study which used the same erosion cycling, O'Toole et al. (2015) compared the protective effects of stannous fluoride and sodium fluoride applications before and after erosion identifying that stannous fluoride reduced step height regardless if it was applied before or after erosion but sodium fluoride only provided protection if applied after erosion. However, in this study step height was recorded from a single line selecting the mid-point in the eroded zone from and calculating the average depth from the two reference areas. Schlueter et al. (2013) used a similar approach to measuring step height in their *in situ* study investigating the anti-erosive effects of a tin-chitosan toothpaste. After experimental erosion-abrasion they scanned their enamel samples using an optical profilometer and on the resulting profile graphs regression lines were added to both reference and eroded areas. The vertical height between these was measured. This was carried out for three profile tracing per sample and averaged. It could be argued that taking a single depth measurement from the mid-point or regression line is not truly representative, the profile tracings in both the eroded and reference areas will not be completely flat. Therefore, the automatic software calculations used by Mistry et al. (2015) and Sancakli et al. (2015) may provide less bias, but taking multiple readings evenly distributed from a scanned area will minimise any bias and provide a robust measurement technique.

Lesion depth measured through profilometry has also been validated against other techniques. Elton et al. (2009) investigated the correlation of measuring lesion depth with non-contact profilometry and transverse microradiography. Transverse microradiography was considered to be the industry gold standard with a reported accuracy of 5 µm for flat surfaces. They identified a strong correlation between lesion depth measurements from both techniques. However, in an aim to explore a technique to measure enamel loss *in vivo* the study also investigated the correlation between Transverse Microradiography (TMR) and quantitative light-induced fluorescence (QLF), both are techniques which can differentiate between sound and demineralised tooth tissues. However, there was a poor correlation between TMR and QLF due to the differences in measurement techniques. This

was disappointing as QLF can be used intra-orally, however replica techniques combined with profilometry could provide an *in vivo* alternative.

1.2.7.3 Replica Techniques

As direct scanning of teeth at such high resolution is not possible, replica techniques can also be used with profilometry and are an important move towards *in vivo* measurement of erosive tooth wear. Hjortsjö et al. (2012) investigated changes in profile roughness and profile height (tissue loss) of enamel samples and their associated replicas. Five enamel samples were prepared for the validation part of their study; the samples were sectioned and one half isolated and the remaining half exposed to citric acid. Impressions were taken of the samples using a light bodied addition cured silicone material (Express II light body, 3M-ESPE) which were then cast in acrylic resin to produce positive replicas. Reference markers were used on the replicas to ensure the same regions were being measured as the enamel samples themselves. Pearson correlation analysis revealed a strong correlation between roughness results for measuring the enamel surface and associated replica and a strong correlation with tissue loss measurements. As well as 2D step height measurements to measure lesion depth 3D techniques that calculate volume loss can also be used. Paepegaey et al. (2013) identified a correlation between 2D and 3D techniques to measure step height. The 3D technique essentially calculated the volume loss by converting the worn area into a cylinder and calculating the radius (from the width of the wear zone) and height (the vertical drop used to calculate 2D step height) (Rodriguez & Bartlett 2010). Measuring the volume of the eroded zone is considered more representative as it accounts for overall changes. These methods can be taken further and used for *in vivo* measurements to attempt to monitor tooth wear progression over time by recording impressions at various time intervals, scanning the corresponding dental casts and using superimposition software to measure any tissue loss which has occurred during the time interval (Ahmed 2014). Rodriguez et al. (2012b) investigated progression of erosive tooth wear of subjects previously clinically diagnosed with tooth wear. They measured profile changes of replica casts with a

NCP and superimposition software to identify changes in tooth profile over a period of 12 months. Dental impressions were recorded using addition cured silicone (Aquasil®, Dentsply, UK) over three visits and cast using dental stone Moonstone® (Bracon Ltd. Etchingham, UK). The materials used had been validated by the authors in previous studies (Rodriguez et al. 2009; Rodriguez & Bartlett 2011). Scans were recorded at baseline and were superimposed and compared to those at 6 months, and those at 6 months were subsequently compared to those at 12 months for comparison. In their analysis, the average wear detected was less than the determined accuracy of the system used. However, they acknowledged that in future studies focusing upon the most severely affected teeth may reduce the measurement error making it easier to identify profile changes over time. Perhaps increasing the duration of the study and recording changes over a three-year period would have also allowed for more changes to have naturally occurred and therefore be detected.

Validation studies have also been conducted to investigate replica techniques that can be used to quantify erosive tooth wear (Whitehead et al. 1997; Ahmed 2014; Rodriguez et al. 2012b). Rodriguez et al. (2009) investigated the innate surface roughness of impression media and dental stone. They identified no differences between measuring the impression media or the dental stone. To prepare the test substrates the stone or the impression media were poured into glass slabs, however the effect of the innate roughness values upon replication surface texture of enamel was not investigated. In a further study to investigate replica substrates, Rodriguez and Bartlett (2011) compared direct profile measurements of a metal block (ADA block) versus impressions of the block taken with 8 investigatory dental materials. There was good correlation between measuring the block versus the replica for each material investigated despite slight contraction where the replicas were concerned. Over a 12-week period the impressions demonstrated dimensional instability which suggests that the replica impressions should be analysed or cast into positive dimensionally stable replicas immediately (Rodriguez & Bartlett 2011). Furthermore, Goodall et al. (2015) carried out a study investigating the use of different impression media for surface roughness of fish teeth. They investigated the effectiveness of replica techniques at measuring rough and smooth surfaces, by comparing the natural

surfaces to the replicas made from the various impression media. They identified good correlation between roughness measurements recorded from the replicas and the true surfaces. However, differences amongst the impression media led them to suggest there is a need for standardisation amongst similar research fields. Despite this they identified a replica technique which was able to replicate the surface texture of enamel and is important for *in vivo* erosion studies.

1.2.8 Microhardness

The origins of the microhardness techniques are based around metals but it has been used to assess enamel for over 50 years (Newbrun 1960). Microhardness testing has been used in erosion studies to investigate changes in surface hardness following exposure to erosive wear and to compare the efficacy of anti-erosion and remineralising products. There are two types of microhardness testers: Knoop and Vickers. The geometry of the two indenters varies and consequently so does their associated algorithms, however the principle remains the same. The software component of the device is used to select a press time and a load onto the surface to be measured and following this an indentation is made the dimensions of which are recorded and used to calculate a hardness number. Knoop indenters penetrate enamel to approximately 1.5 µm whereas Vickers to 5 µm leading some authors to suggest that Knoop indenters are more accurate when identifying early erosive tooth wear (Schlueter et al. 2011). Although this is refuted in a more recent study by Lippert and Lynch (2014) which showed no difference between Knoop and Vickers at measuring hardness change after early or more pronounced demineralisation. They investigated changes in lesion length and lesion depth of Knoop and Vickers indentations on polished human and bovine enamel samples at baseline and following demineralisation to create early caries lesions. They recorded the length of the microhardness at baseline and after demineralisation and used microradiography to measure the depth of the indentation lesion and mineral analysis. Whilst finding no differences between the two types of indenter both demonstrated a strong correlation between length of the indentation lesion and the depth suggesting that microhardness measurements are successful in identifying changes in

enamel structure. The press and load time selected and the type of surface being measured must also be taken into consideration. Chuenarrom et al. (2009) compared press time and load for both Vickers and Knoop indenters when measuring surface hardness of enamel and dentine. They reported that press time did not exert any change on the measurement values for both types of indenters for both enamel and dentine but altering the load had an effect on values for Knoop of enamel and for Vickers on dentine. The accuracy of the results may be questionable. It is standard practice to leave 100 µm between each indentation when using Knoop indenters, and 150 µm for Vickers (Lippert & Lynch 2014). The samples used by Chuenarrom et al. (2009) were each indented 27 times in total. Each enamel sample tested was only approximately 2 by 2 mm, therefore the close proximity of the indentations could have affected the accuracy of their measurements. Whilst smooth flat and shiny surfaces remain the optimum properties for measuring microhardness, there have been limited endeavours for its use *in vivo*. Therefore, it is normally applied for *in vitro* or i*n situ* studies using polished enamel or dentine samples (Schlueter et al. 2011). The level of erosion studied can also affect the accuracy of microhardness testing as the outlines of the indentations are much more difficult to identify on increasingly eroded surfaces. Stenhagen et al. (2010) compared various methods of analysis of erosion and progression, and found microhardness to be the least reliable suggesting microhardness should be used primarily for early lesions where there has been no bulk surface loss. Lussi et al. (2000) identified significant reduction of surface hardness of both deciduous and permanent enamel samples *in vitro* after only three minutes erosion exposure to various commercially available acidic soft drinks. Microhardness has also been used to determine the effects of anti-erosive agents on early erosion-abrasion lesions. Carvalho and Lussi (2014) measured microhardness change of enamel samples and calculated substance loss by calculating the difference in length and depth between baseline and subsequent microhardness indentations following erosion-abrasion. There is a constant ratio between the length and depth of indentations making this a robust and reproducible method of calculating surface changes as well as providing a cost effective means as both hardness change and surface change are capable of being calculated from the same indentations. Studies which

use only the length of the indentation lesions have been criticised as acid removes tissue from the body and not only the periphery (Attin 2006). Carvalho and Lussi (2014) investigated the difference between control samples and samples brushed using a sodium fluoride toothpaste, samples brushed using stannous fluoride chitosan toothpaste, samples brushed using a sodium fluoride toothpaste plus a sodium fluoride rinse and samples brushed using stannous fluoride chitosan toothpaste plus a stannous fluoride chitosan rinse. Each erosion-abrasion cycle consisted of immersion in the designated toothpaste for 2 minutes, toothbrush abrasion for 10 seconds, immersion in citric acid for 2 minutes and completed by rinsing in water or the test solutions this was repeated once a day and completed for 8 days in total. They identified that microhardness change occurred after 1 cycle for all experimental groups. This highlights the benefits of using microhardness testing to identify early changes following erosive tooth wear.

However, studies which aim to combine microhardness tissue loss often use profilometry and hardness testing. Hara and Zero (2008) compared surface microhardness change on polished bovine enamel samples. They compared the effects of immersing the samples in 10 different beverages after 0, 5, 10, 30, 60, and 120 minutes analysing for microhardness change and profilometry. They identified significant softening of enamel after 5 minutes of erosion but longer erosion time was required for profilometry. Therefore, when studies combine profilometry and microhardness testing erosion times will be longer as a minimum lesion depth of 0.3 µm is required to detect tissue loss (Scaramucci et al. 2011). Scaramucci et al. (2011) measured profile and microhardness of bovine enamels samples which had been immersed in modified acid beverages for 30, 90 and 150 min. Whilst the surface hardness became softer with increased immersion time, increased variability within the sample groups is apparent. It is accepted that enamel becomes softer following erosion and hardens with remineralisation (Burwell et al. 2009; Zhou et al. 2012; Joiner et al. 2014; Nehme et al. 2016; Hara et al. 2009). This has been utilised to determine 'hardness recovery' and used as a quantitative tool to express this data. Hara et al. calculated the percentage surface microhardness % SMR recovery from

the lengths of five indentations recorded at baseline (Lb) after erosion (Ld) and after remineralisation (Lr) as shown in Equation 1 below.

$$\%SMR = 100\ x\ \frac{Ld - Lr}{Ld - Lb}$$

Equation 1: Equation used to calculate %SMR

The higher values reflect higher recovery, indicating the efficiency of the remineralisation product (Hara et al. 2009). In contrast however, a recent study identified that immersing enamel samples in saliva before erosion cycling significantly increased softening of the enamel (O'Toole et al. 2015). They suggested this may be therapeutic as although the enamel is softened bulk tissue loss is lowered thus suggesting that less enamel damage has occurred.

1.2.9 Scanning Electron Microscopy

Scanning electron microscopy (SEM) originates from 1926. It works by emitting a probe of electrons onto the surface of a specimen and scanning horizontally. The signals generated on the impact of the electrons are used to construct an image of the surface or to characterise the specimen (Bogner et al. 2007). Conventional SEM requires coating the specimen with an electrical conductive material such as gold (Field et al. 2010). This can be disadvantageous as the specimens are irreversibly changed and cannot undergo further investigations as well as being costly. This has been overcome with the development of environmental SEM (ESEM), which introduced gas into the specimen chamber, meaning that the specimens are in a low-pressure environment as opposed to the high pressure of conventional SEM. The gas acts as an electrical conductor removing the necessity for gold coating (Bogner et al. 2007). The high-resolution images produced from SEM and ESEM can provide qualitative data. However, studies investigating enamel erosion often assign a grading scale to assess the images to produce quantitative data, but this is highly subjective (Attin & Wegehaupt 2014). An advantage of

SEM imaging is that it can be used on enamel specimens or replicas of enamel specimens and therefore can be used as a technique to investigate clinical progression of erosive tooth wear. When SEM imaging of a specimen is combined with energy-dispersive X-ray detection (EDX) it is possible to record the mineral content of the specimen examined providing 'true' quantitative data (Worobiec et al. 2010). Coceska et al. (2016) investigated the remineralising ability of four toothpastes using SEM and EDX. SEM images were used for qualitative assessment and EDX used to quantify the percentage of sodium, magnesium, aluminium, phosphorus and calcium present in the specimens. Limandri et al. (2016) combined stereo SEM with specialised software to transform the SEM images into topographical maps from which roughness parameters were calculated. They investigated the surface of teeth following whitening procedures versus controls. The samples were imaged using SEM with functional and amplitude parameters extrapolated from the height maps obtained for stereo image pairs. They compared the results achieved with those recorded from gold standard CLM measurement. The close agreement of the two suggested a comparable and reliable method to achieve qualitative and quantitative interpretation of the underlying surface topography.

Overall, SEM is an established reliable method to examine structures at high resolution and can be used in combination with other tools to provide quantitative as well as qualitative data with further developments being investigated to combine SEM, EDX and Raman spectrometry (Limandri et al. 2016; Worobiec et al. 2010; Coceska et al. 2016).

1.3 Variables in models to investigate erosive tooth wear

Huysmans et al. (2011) commented that existing *in vivo* methods remain unable to accurately measure early erosive changes to enamel and acknowledged that extensive research was needed to develop better techniques to investigate if early structural changes, such as surface roughness, could be used clinically. It has been suggested that laboratory studies overestimate erosive changes by 10 times (Barbour & Rees 2004). *In situ* studies have the added advantage of being able to utilise gold standard *in vitro* techniques, including microhardness and profilometry, to identify structural changes in enamel

with a more real-life environment involving saliva and pellicle formation as well as behavioural influences. Although saliva and pellicle formation can be incorporated in i*n vitro* studies, it is accepted that *in situ* studies provide a closer representation to the clinical scenario.

1.3.1 Saliva and the acquired pellicle

The salivary pellicle (also referred to as acquired pellicle) is a thin biofilm that contains proteins, glycoproteins, mucins and enzymes and coats the surfaces of teeth in a natural oral environment, and is believed to limit the demineralisation from acid erosion (Lendenmann et al. 2000; Hannig et al. 2005a). Hannig et al. (2005a) attributed this protective effect to the carboanhydrase species of enzymes within the pellicle. Hannig (2007) compared the protective effect of salivary pellicle formed over 3, 60 and 120 minutes *in situ*. Healthy volunteers wore splints containing bovine enamel samples for either 3, 60 or 120 minutes to allow for pellicle formation. The bovine samples underwent an *ex vivo* immersion in 1% citric acid for 60 seconds. Microhardness measurements were recorded before and after erosion and enamel samples without pellicle formation were investigated in the same way. Their results showed no difference in the protective effect between pellicle formed over 3, 60 or 120 minutes suggesting pellicle formation after 3 minutes provides adequate protection from erosion. However, whilst a protective effect is achieved by the pellicle it does not provide complete resistance to erosion. Nekrashevych and Stösser (2003) compared roughness, microhardness changes and calcium release of polished bovine enamel in dentine with or without pellicle protection. The samples which had a salivary pellicle were immersed in collected saliva *ex vivo* for 24 hours. Samples were either eroded in 0.1 % or 1.0 % citric acid for 1, 5 or 10 minutes. Enamel samples with pellicle had significantly less microhardness change following erosion with the exception the 10 minutes immersion in 1.0 % citric acid. With regards to calcium release there was no significant difference between the pellicle covered and the non-pellicle groups. However, roughness changes were significantly less for the pellicle covered samples and qualitative observations from SEM images also suggested a protective effect up to 10 minutes of erosion. Furthermore, Hara et al. (2006b) suggested

that the protective effect of the pellicle may be reduced or removed after 10 minutes of erosion. To allow the pellicle to form participants wore a palatal appliance containing polished enamel and dentine samples for two hours versus control groups without pellicle formation. They measured surface hardness changes of the enamel samples and lesion depth and mineral loss of the dentine samples with and without pellicle formations. They measured changes after 0, 10, 30 minutes of erosion. After 10 minutes of erosion there were no statistical differences between enamel groups suggesting protection for only the first 10 minutes. Whereas the pellicle offered no protection for dentine samples. However, caution with interpreting these results must be taken as there was only one outcome measurement for enamel, microhardness. Microhardness measurements become less reliable with increased erosion. Furthermore, Moazzez et al. (2014) investigated the protective effects of saliva pellicle of individuals with erosion compared to those without. An *in situ* model was used to acquire salivary pellicle on enamel samples from 30 participants diagnosed with erosive tooth wear and 30 healthy volunteers, a further 30 samples were prepared and not exposed to an oral environment. All the enamel samples were immersed in citric acid *ex vivo* and surface roughness, step height and microhardness were measured before and after erosion. The surface roughness and microhardness results showed a difference in the protective effect between salivary pellicle of erosive wear patients. It has also been suggested that the salivary pellicle of adults and children have different protective effects (Carvalho et al. 2016a). Whilst *in situ* studies utilise the formation of the pellicle to investigate its protective effects, to be able to truly investigate surface or structural changes of the enamel sections during the study, the pellicle must first be removed before any post experimental profilometric or microhardness investigations. Hannig et al. (2005b) compared different methods used to remove the pellicle shown in Table 7.

Table 7: Effectiveness off different methods used to remove the salivary pellicle (Hannig et al. 2005b).

Treatment of the *in situ* formed pellicle layer and ultrastructural findings (appearance of the residual pellicle layer)		
Treatment mode and time	**Ultrastructural appearance of the buccally formed 2-h pellicle**	**Ultrastructural appearance of the palatally formed 2-h pellicle**
No treatment (control samples)	Electron dense, 10–20 nm thick basal layer covered by a 100–300 nm thick globular layer	Electron dense, 10–20 nm thick basal layer covered by a 20–50 nm thick granular layer
0.6 M hydrochloric acid, 40–180 s	Complete removal of the pellicle	Complete removal of the pellicle
0.4% EDTA (pH 7.4), 20 min	No alteration of the pellicle	No alteration of the pellicle
0.4% EDTA (pH7.4), 40 min	Pellicle residues	Pellicle residues
0.4% EDTA (pH 7.4), 60 min	Complete removal of the pellicle	Complete removal of the pellicle
Phosphate buffer solution (pH 7.4), 24 h	Partial removal of the globular layer	Partial removal of the granular layer
15% sodium chloride, 60 min	Partial removal of the globular layer	Partial removal of the granular layer
2 M calcium chloride, 60–90 min	Partial removal of the globular layer	Partial removal of the granular layer
1 M sodium thiocyanate, 20–60 min	Partial removal of the globular layer after 60 min	Partial removal of the granular layer
2% urea, 20–60 min	Partial removal of the globular layer after 60 min	Partial removal of the granular layer
5% tetrahydrofurane, 20–60 min	Partial removal of the globular layer after 60 min	Partial removal of the granular layer
6 M guanidine hydrochloride, 24 h	Nearly complete removal of the globular layer, no alteration of the basal layer	Partial removal of the granular layer, no alteration of the basal layer
2% sodium dodecyl sulfate, 60 min	Partial removal of the globular layer, no alteration of the basal layer	Partial removal of the granular layer
Scraping with scaler/curette	Removal of the globular layer, partial disruption of the basal layer	Removal of the granular layer, intact basal layer
Scraping with razor blade	Nearly complete removal of the globular layer, partial disruption of the basal layer	Partial removal of the granular layer, intact basal layer
Rubbing with a plastic foam sponge (Pele Tim; Voco,	Removal of the globular layer	Removal of the granular layer

Cuxhaven, Germany) containing 5 µl water		
Rubbing with a plastic foam sponge (Pele Tim; Voco, Cuxhaven, Germany) containing 5 µl 2% sodium dodecyl sulfate	Removal of the globular layer	Removal of the granular layer
Water, 30-min ultrasonication	Removal of the globular layer, intact basal layer	Partial removal of the granular layer, intact basal layer
6 M guanidine-hydrochloride, 30-min ultrasonication	Residues of the basal layer	Residues of the basal layer
2 M calcium chloride, 30-min ultrasonication	Removal of the globular layer, intact basal layer	Removal of the granular layer, intact basal layer
15% sodium chloride, 30-min ultrasonication	Removal of the globular layer, partial disruption of the basal layer	Removal of the granular layer, intact basal layer
3% sodium hypochlorite, 30-min ultrasonication	Complete removal of the pellicle	Complete removal of the pellicle

As well as protection from formation of the acquired pellicle saliva influences erosion potential in its buffering ability to neutralise acids, remineralisation effect as well as a physiological ability to remove residual debris from the tooth surfaces. The salivary pH is known to influence buffering with an increased salivary pH able to buffer acids and allow for remineralisation of tooth structures to occur, saliva contains variable amounts of calcium, phosphate and fluoride which are responsible for its remineralising effect (Loke et al. 2016). Buffering capacity is commonly linked with saliva flow rate, with reduced saliva flow adversely affecting its buffering ability (Buzalaf et al. 2012). Using *in vitro* studies to develop a technique for early quantification of structural changes in enamel and then utilise these techniques *in situ* are imperative in the development of method that could be used to clinically identify early erosive tooth wear.

1.3.2 *In situ* Appliances

West et al. (2011) broadly described the types of appliances used in *in situ* studies, as removable appliances which can be worn intermittently or continually and fixed appliances for continual use. They suggested a removable appliance worn during office hours under supervision enhances compliance as behavioural habits such as smoking etc. can be monitored. One research group conducted a series of studies establishing a method to investigate the erosive effect of a modified beverage using the same appliance design (West et al. 1999; Hughes et al. 1999b; Hughes et al. 1999a). A removable maxillary acrylic appliance with molar clasps and a palatal recess area to house an enamel sample was worn from 9 am to 5pm of working days for a total of 15 days. The devices were removed for an hour at lunchtime and oral intake whilst wearing the devices was restricted to tea, coffee or water. Consumption of the investigated beverages was conducted at set time intervals and durations under supervision. This type of supervised regime reduces participant variations due to compliance issues and time management errors of an individual resulting in concise documentation in the study protocol. However, it is limited in that it does not allow for a 'real life' environment that accounts for overnight exposure for example. In contrast studies, which require continuous wearing of the

appliance may allow for appliance removal during eating, drinking, oral hygiene etc. and these intervals may not be consistent amongst a study group. However, 24-hour exposure to the oral cavity is necessary to assess remineralisation which takes priority in these studies (Conceição et al. 2015). Mathews et al. (2012) used a fixed appliance approach by cementing customised orthodontic molar pads with retentive mesh backing, which had a stainless steel band welded to tightly hold an enamel block. The enamel block was secured within the bracket using a fluoride-free Intermediate Restorative Material (DENTSPLY Caulk, Milford, DE USA). The study lasted 28 days, in this case a fixed appliance reduced compliance issues. Despite this 2 out of their 20 participants were withdrawn for compliance issues to the test products. A fixed appliance may seem an obvious solution for longer *in situ* studies. However, debonding can be an issue and there is the possibility of damage to the participants own oral health through increased plaque retention. Furthermore, prolonged studies will have compliance issues regardless of the type of appliance used.

Another consideration with appliances is their location with both maxillary and mandibular splints routinely used for *in situ* studies. Hooper et al. (2007) compared tissue loss of human enamel samples positioned in the anterior third of the palate compared to samples positioned in the posterior third of the same participants wearing a single appliance fitted with two enamel samples in the described locations. There were no statistical differences between results from either location. Although it is not always stated why a particular design is used it could be argued that mandibular devices allow more saliva to accumulate as saliva naturally gathers in the floor of the mouth therefore, results may not be comparable with a study which used a palatal appliance. Erosion is common on the palatal surfaces of upper incisors but rare on the lingual surfaces of lower incisors, which has been linked to the increased presence of saliva (Hara et al. 2006a). Amaechi and Higham (2001a) investigated the remineralising effects of saliva relating to tooth location in an *in situ* study. Twenty pre-eroded enamel samples were prepared (2 samples per collected tooth). Ten participants were recruited and fixed appliances were used to house one sample in the upper arch (positioned on the palatal incisor) and one sample in the lower arch. The appliances containing the samples were worn for a total of 28 days during which, with

exception of chewing a sugar free gum four times a day, the participants continued with their usual oral and dietary habits to investigate the natural remineralising process. The enamel samples which had been located on the lower lingual surfaces demonstrated significantly less mineral loss and lesion depth suggesting a better remineralising effect. However, when samples were positioned buccally in the lower arch there was the same level protection as exhibited palatally. Mendonça et al. (2017) recently compared the use of palatal and mandibular appliances when measuring microhardness change of bovine enamel following *ex vivo* erosion in hydrochloric acid for 30 seconds. The maxillary appliance had a palatal vertical channel on both sides with a recess area to house an enamel sample, totalling two samples per device. For the mandibular, two appliances were made each with a recess area buccally to retain an enamel sample, totalling two samples per participant. Both appliances were constructed of acrylic and used orthodontic wires to protect the samples from soft tissue abrasion from cheek or tongue. Their results suggested no difference in microhardness change using either appliance. Whilst this study is by no means exhaustive, as it does not take in to account roughness changes, tissue loss or mineral analysis it would suggest that either palatal or mandibular buccal placement of samples can be considered representative of regions that are naturally prone to erosive tooth wear. Non-scientific considerations also have a place when deciding upon appliance design such as comfort, cost and safety. Ensuring samples are secured to reduce the risk of swallowing and inhaling supports the use for of mandibular designs with buccally positioned samples. Soft vacuum-formed splints may be more comfortable and applicable for short term use whereas for studies where the appliance is to be worn for considerable durations a more robust design in hard acrylic may be preferred (Bartlett et al. 2003; Conceição et al. 2015; West et al. 1999; Hughes et al. 1999b; Hughes et al. 1999a).

1.3.3 Erosive *in situ* regimes

In situ studies can use *ex vivo* erosion cycling or they can use *in vivo* erosion cycling. *Ex vivo* includes to use of citric acid, hydrochloric acid or consumable acid such as grapefruit juice to create erosive

lesions prior to inserting the samples into an appliance and investigating remineralising properties (Conceição et al. 2015; Nehme et al. 2016; Hannig et al. 2007). *In vivo* erosion cycling is limited in the extent of erosion investigated, due to ethical considerations, but provides a more clinically representative investigation of the natural intra oral environment and salivary benefits. Scaramucci et al. (2012) investigated erosive potential of an orange juice modified with calcium and/or linear sodium polyphosphate versus the orange juice itself. Polished bovine enamel samples were used and %SMC used as the outcome measure. Healthy volunteers (n=10) wore palatal splints containing 8 bovine enamel samples and performed erosive challenges for a total of 0 (control), 10, 20, and 30 minutes. Each volunteer took 10 mL of their designated juice into their mouths and held the liquid against the palatal appliance with their tongue for 15 seconds, expectorated the juice, waited for 15 seconds, and repeated the procedure continuously 40 times resulting in 10 minutes of direct exposure to 400 mL of the juice. After completing one cycle two specimens were removed from the device during and the erosion cycling repeated removing two specimens after each cycle until 0, 10, 20 and 30 minutes of erosion had been completed. Therefore, the maximum erosion the volunteer experienced was 30 minutes of total exposure to 1.2 l of the juice. West et al. (2011b) conducted a study where participants sipped 250 mL of orange juice over a 10-minute period four times a day for a total of 15 days with no adverse events recorded. However, Nehme et al. (2016) created erosive lesions on bovine enamel samples *in vitro* and used palatal splints to investigate the remineralising properties of toothpastes. The appliances were either worn for a total of four or eight hours. The subjects wore a palatal appliance holding six (4-hour group) or eight (8-hour group). After an initial 5 minutes wearing the splint for equilibrium they were then asked to brush the facial surfaces of their natural teeth with the test toothpaste for 25 seconds and then swish the slurry around their mouth for 1 minute to allow direct contact with the enamel specimens. After expectorating the slurry, the subjects rinsed their mouths with 15 mL of water for 10 seconds. This was carried out under supervision and afterwards they continued to wear the splint for 4 or 8 hours depending on their group allocation. By supervising the brushing and rinsing this helped standardise the study. Since it is the remineralising products that

were of interest rather than the demineralising process it is appropriate that the erosive lesions were created *in vivo* as these would be more consistent and standardised than *in vivo* demineralisation. The remineralising products were commercially available and therefore FDA approved. This is ethically significant as investigating products such as toothpastes is classed as a CTIMP therefore must be safe for the subjects involved. Conceição et al. (2015) also used an *in vitro* approach to create erosive lesions. Appliances were worn containing four enamel specimens for 2 hours to allow for pellide formation. One side was allocated for erosion only and the other for erosion-abrasion. Specimens were then pre-treated with investigatory anti erosive products (versus control) *ex vivo*, immersed in citric acid for 5 minutes four times a day for a total of five days *ex vivo* and followed or not by abrasion. The 30 seconds abrasion was performed by the volunteers after each erosive challenge using a soft bristle toothbrush for half the specimens whereas the remaining specimens were immersed in a toothpaste slurry during this time. This study combined *in vivo* and *ex vivo* approaches but it is not always clear why. If using *in vitro* erosion to produce a standardised lesion it would follow suit to use the same approach for the erosion abrasion lesions and use a standardised tooth brushing machine. Perhaps this was not readily available to the authors, explaining the *in vivo* design of this phase of their study. The benefits of the *in situ* nature of this study are to investigate the effect of the salivary pellicle and the remineralising product, which was applied *ex vivo*. Applying the product *in vivo* would have provided a more realistic effect as the effect of intra oral saliva flow upon the efficacy of the products were not investigated in this study. Overall to achieve a study that investigates natural erosive changes an *in vivo* approach should be used as much as possible.

Overall Aims and Objectives

The overall aims and objectives of the thesis were to:

- Develop a method to quantify surface roughness of natural unpolished enamel which may be adapted for *in vivo* use to diagnose and predict progression of erosive tooth wear. At the beginning of this thesis surface roughness measures on natural unpolished enamel were limited to a small *in vivo* study (Whitehead et al. 1997).
    - To develop an erosion model that can successfully identify changes in topography of both polished and natural unpolished enamel.
    - To assess the feasibility of microhardness measurements on natural unpolished enamel surfaces.
    - To compare surface roughness measurements with industry gold standards, step height and microhardness.
    - To assess the effect of saliva on surface roughness changes, step height and microhardness change using an *in situ* model.

Chapter 2 Development of a laboratory method to quantify surface changes of natural unpolished enamel following dietary erosion

2.1 Introduction

There has been an increase in the prevalence of erosive tooth wear (White et al. 2012). General attitude and perception towards erosive tooth wear has also changed, with dentists being more clinically aware (Lussi & Carvalho 2014). This has led to an increase in research to identify the earliest signs of erosive tooth wear, which is described as a loss of surface texture (Bartlett et al. 2008). Surface roughness measurements have become increasingly used in dental material science and have been advocated for early quantification of erosive tooth wear (Austin et al. 2015; Joshi et al. 2016). Surface roughness measurements have been successfully used in erosion studies with polished enamel samples to investigate changes following erosion and subsequent remineralisation (Austin et al. 2016; Gracia et al. 2010; Zhou et al. 2012). However, there is limited work using natural unpolished enamel samples. Furthermore, it is not possible to use the high-resolution equipment required to measure surface texture of enamel intra orally, meaning replica methods must be explored for *in vivo* studies.

This Chapter reflects the early stages in developing a method to quantify surface roughness changes of natural unpolished enamel and polished enamel using a red laser confocal profilometer. As the method developed and the device which would be validated in this thesis were both novel, early work also utilised gold standards such as confocal laser microscopy, microhardness testing and white light profilometry for step height measurements (Schlueter et al. 2011; Austin et al. 2016; Mistry et al. 2015).

There were six studies completed in this chapter. The first used a high resolution and traceable CLM to investigate the effects of increasing erosion times on surface roughness of natural unpolished enamel. Whilst access to this device was not available after initial work, its use provided the initial background to further develop a method for identifying changes in surface roughness of natural

unpolished and polished enamel using the red laser confocal profilometer. Study 2 investigated feasibility of measuring microhardness of natural unpolished enamel. Microhardness is considered to be the gold standard for studies investigating early erosion using polished enamel samples, but studies using natural unpolished enamel samples are limited. Study 3 investigated the feasibility of the use of reference barriers with unpolished natural enamel. Reference barriers are required to measure step height change in accordance to ISO standards 5436. However, it was unknown what effect these may have on surface roughness measurements. In Study 4 the polishing protocol was reviewed with regards to its efficiency when surface roughness was an outcome measure. In Study 5 the erosive potential of two orange juice products and their effects on surface roughness of natural unpolished enamel were investigated. Finally, in Study 6 a replica technique was investigated to determine if the methods developed for quantifying changes in surface roughness of natural unpolished enamel could be implemented *in vivo.*

2.2 Aims

The overall aim of Chapter 2 was to develop a method to enable changes in natural unpolished enamel and polished enamel following erosion from dietary acid to be measured, through a series of pilot studies.

- Study 1: to quantify changes in Sa roughness of natural unpolished enamel following exposure to dietary acid using a high resolution and traceable CLM.
- Study 2: to assess the feasibility of microhardness testing on natural unpolished enamel.
- Study 3: to investigate the effectiveness of different reference barriers on natural unpolished and polished enamel samples.
- Study 4: to investigate the addition of diamond polishing paste to existing protocols for polishing enamel samples.
- Study 5: to compare the effects of two orange based beverages on Sa roughness of natural unpolished enamel.

- Study 6: to develop a replica technique to measure Sa roughness of natural unpolished enamel *in vivo.*

2.3 Study 1

2.3.1 Methods

2.3.1.1 Sample preparation

To prepare enamel samples used throughout this thesis caries free human molars, which were scheduled for extraction for clinical reasons, were collected following written consent from patients in the oral surgery department at King's College London (KCL) Dental Institute under ethical approval (REC reference: 12/LO/1836, Bloomsbury). The collected teeth were stored in sodium hypochlorite for a minimum of three days following KCL Health and Safety recommendations. The roots were removed and the crowns sectioned using a water cooled circular diamond saw (XL 12205, Benetec Ltd., London, UK) at a speed of 600 rpm. Each tooth was secured in a holder using greenstick impression compound (Kerr™ Corporation, USA) and positioned perpendicular to the saw for decoronation as shown in Figure 14. The crown was then further divided to separate the buccal and lingual halves. The buccal section was further sectioned in half to produce 5 mm x 8 mm x 2 mm sections of enamel (width, depth and height) shown in Figure 15. Five natural unpolished enamel samples were prepared from buccal sections and embedded in cold cure acrylic (Oracryl Bracon Ltd, UK) using a customised mould tray and leaving the outer surface uncovered, as shown in Figure 16. Following which they were inserted into an ultrasonic bath (Nusonics GP-70, T310) set at 60 Hz for 15 minutes, for cleaning prior to erosion and imaging.

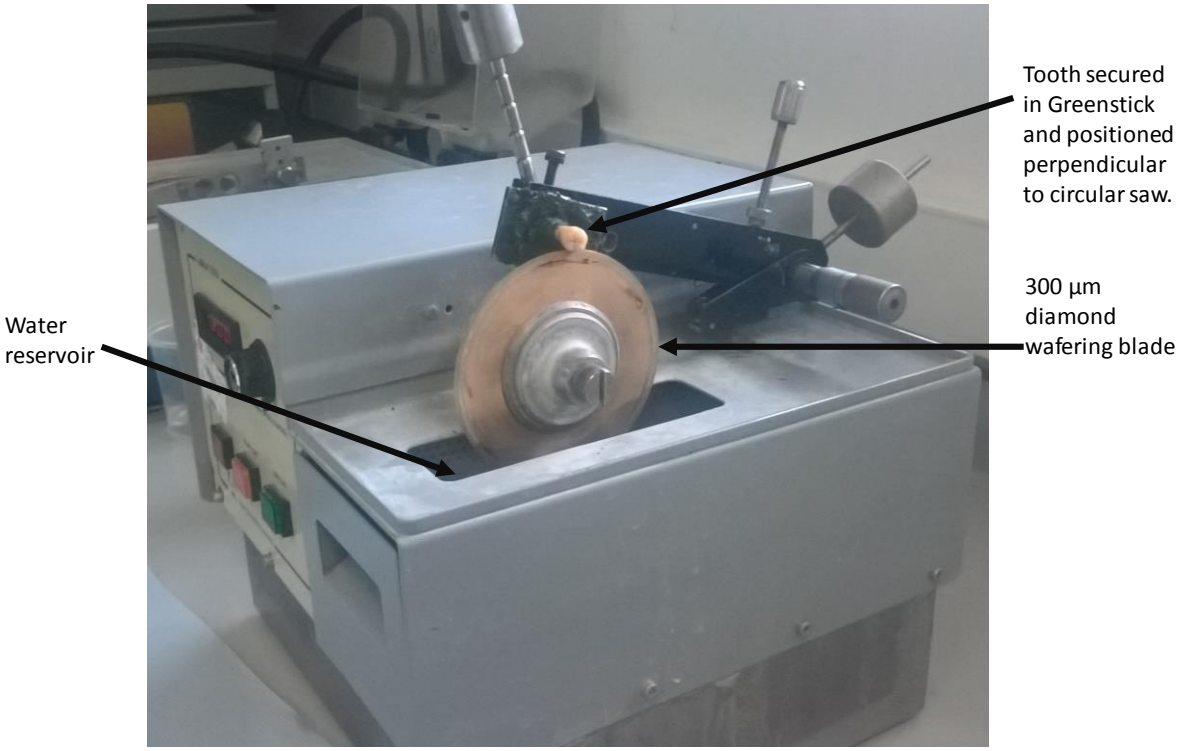


Figure 14: Each tooth was secured in a holder using greenstick and positioned perpendicular to the saw for decoronation.

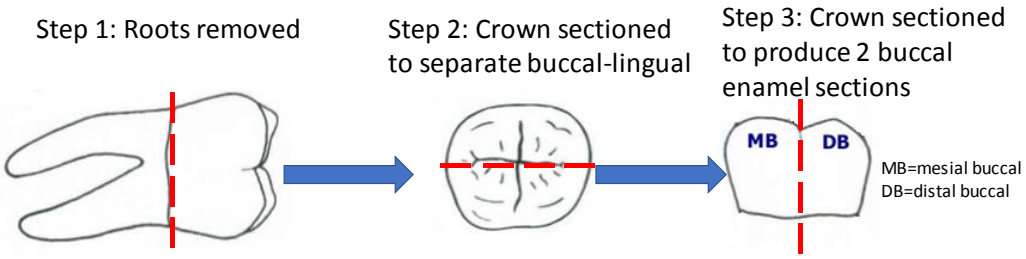


Figure 15: Schematic demonstrating the sectioning process to produce two buccal sections of enamel per tooth.

Figure 16: Photograph of completed natural unpolished enamel.

2.3.1.2 Erosion regime

The five samples were immersed for a total of six-cycles of erosion. For each cycle the five samples were immersed in 50 mL of commercially available orange juice pH 3.9, titratable acidity 110.5 mmol/L (Sainsbury's Basics Orange Juice, Sainsbury's Supermarkets Ltd, London). The samples were eroded under constant agitation at 62 rpm (Stuart Scientific, Mini Orbital Shaker S05, Bibby) for 10 minutes and between cycles the samples were rinsed with deionised water by spraying the samples from a water bottle held approximately 5 cm from the samples for 30 seconds (Mistry et al. 2015) and repeated 6 times shown in Figure 17.

After each cycle the samples were air blasted dry and imaged using a CLM. To ensure all five samples were removed from the solution simultaneously a customised basket was used, the samples were positioned enamel surface facing upwards as shown in Figure 18.

Figure 17: Six- cycle erosion regime used in Chapter 2 Study 1.

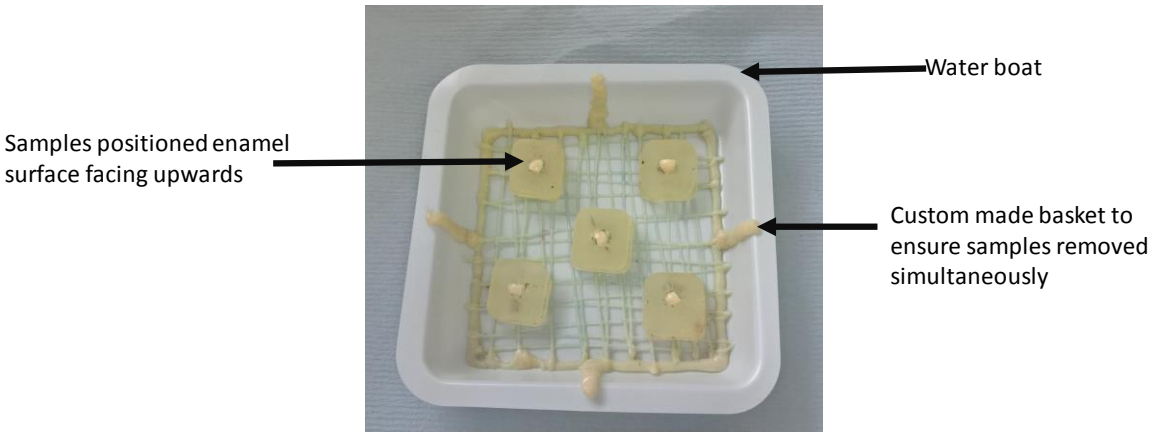


Figure 18: Basket used to insert and remove enamel samples in the erosion liquid simultaneously.

To measure the pH of the solutions firstly two buffers were prepared to calibrate the pH Meter (Oakton pH 510 bench top meter). One pH 4 buffer tablet was crushed and dissolved in 100 mL of deionised water in a clean volume flask using a magnetic stirrer and same procedure used for a pH 7 buffer tablet. To calibrate the pH meter the calibration mode was selected, the electrode rinsed with distilled water and inserted into the buffer, once the reading stabilised enter mode was selected to end the calibration process. This was repeated for the pH 7 buffer. Following calibration of the pH meter the pH of the orange juice was recorded. Measurement mode was selected and the electrode rinsed and inserted into 100 mL of the orange juice and the reading was recorded once it had stabilised. This was repeated three times and the average calculated to one decimal place.

To measure titratable acidity (TA) of the orange based beverages 0.05 M sodium hydroxide buffering solution was prepared by weighing out 1 g of sodium hydroxide powder using an electronic analytical

scale (Mettler Toledo, XS105 Dual Range Analytical Balance) and dissolving it in 500 mL of deionised water in a clean volume flask using a magnetic stirrer. Once prepared, the sodium hydroxide was placed into a clean burette for titration. A pipette was used to obtain 10 mL of the orange juice and placed in a clean beaker positioned below the burette tip with the pH electrode position in the beaker of solution and beaker on top of a magnetic stirrer. The sodium hydroxide was titrated 1 mL at a time and the pH of the orange juice checked after a two minute wait period. This was repeated until the pH reached 7. The whole process was repeated until there was agreement between two readings for the sodium hydroxide solution to within 0.5mL of each other. The following equation (Equation 2) was then used to calculate the titratable acidity in mmol/L where Cbase is the concentration of the base in mol/L, Vbase is the volume of base required to raise the solution to the end point pH in L and Vsample is the volume of the sample that was titrated in L.

$$\boldsymbol{mmol/L = \frac{Cbase\ x\ Vbase}{Vsample}}$$

Equation 2: Equation used to calculate the titratable acidity in mmol/L where Cbase is the concentration of the base in mol/L, Vbase is the volume of base required to raise the solution to the end point pH in L and Vsample is the volume of the sample that was titrated in L.

2.3.1.3 Image acquisition

Images were acquired using a confocal laser scanning microscope (CLM, LEXT OLS4100, Olympus, Tokyo, Japan) at the Division of Engineering Nanometrology at the National Physical Laboratory (NPL, Teddington, UK). The CLM had a 0.2 µm spot size, and reported angular tolerance of 85 % and vertical resolution of 10 nm, shown in Figure 19.

Figure 19: Confocal Laser Microscope LEXT OLS4100, Olympus, Tokyo, Japan).

Images were acquired at baseline and after 10, 20, 30, 40, 50 and 60 minutes of erosion. The CLM emitted a white light onto the measured surface through a confocal aperture. Once optimum focus was achieved the sample was scanned in a raster pattern with the light source moving in the x & y axes. To form a raster pattern an area is scanned from side to side in lines from top to bottom and signals returned from the focus points over the surface to the internal detector providing distance data for each point as shown in Figure 20. Only surfaces within the focal plane provided valid data signal and a 3D image was built up line by line across the overall measured surface. Five representative areas were selected in the centre of each sample, each 129 µm × 129 µm, and scanned using a 20x objective with a 2x optical zoom for each erosion time following previously published protocols (Austin et al. 2016).

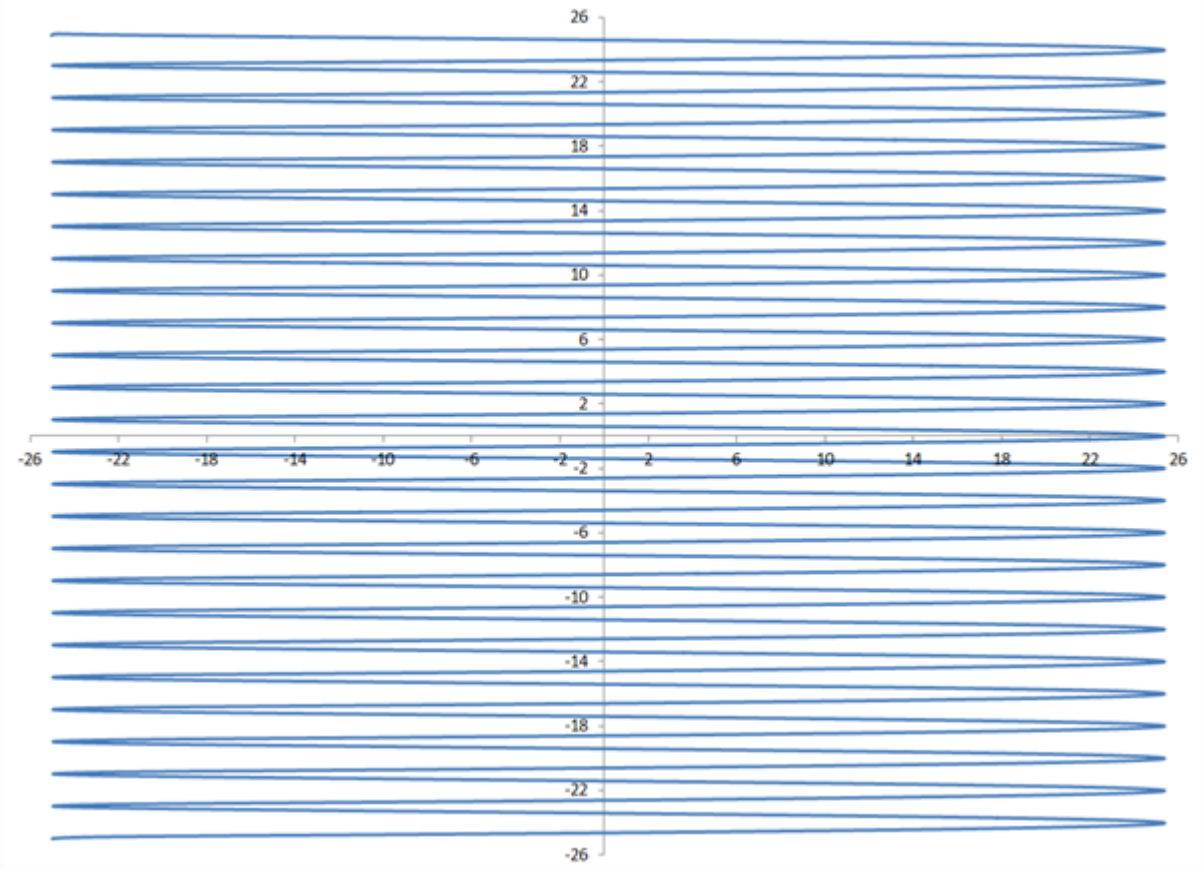


Figure 20: Schematic of traditional raster pattern. Lines represent X vs. Y sensor data for a 40 X 50 micron section of the 40 Hz triangle raster scan (nPoint 2017).

The scans were analysed to calculate 3D surface roughness (Sa) using surface metrology software (MountainsMap, DigitalSurf, France). First, they were levelled; the form was removed and then a low pass 1 µm filter and a high pass filter of 30 µm were applied to extract the roughness wavelengths from the waviness, which was then expressed as Sa (µm) (Leach 2014). Each scan also produced a visual image of the scan area and three representative images were conveniently selected, of the same sample, at baseline, 10 minutes and 60 minutes of erosion to represent the images seen across all scans for qualitative assessment as shown in Figure 21.

2.3.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA). Histogram plots and Shapiro Wilk studies were used to determine normality. The data were normally distributed and a repeated measure ANOVA was conducted to analyse the data with significance set at $p<0.05$. Mean and Standard Deviation Sa roughness values were calculated from the 25 scan areas at baseline and after 10, 20, 30, 40, 50 and 60 minutes.

2.3.2 Results

There was a mean (SD) surface roughness (Sa) at baseline of 0.270 (0.110) µm and following immersion for 10, 20, 30, 40, 50 and 60 minutes of immersion in orange juice there was a statistically significant decrease in mean (SD) Sa roughness values to 0.150 (0.062) µm ($p<0.01$), 0.125 (0.033) µm ($p<0.001$), 0.146 (0.067) µm $p<0.01$, 0.117 (0.028) µm ($p<0.0001$), 0.120 (0.053) µm ($p<0.0001$), 0.117 (0.032) µm ($p<0.0001$) respectively as shown in Table 8.

Table 8: Study 1. Mean (SD) Sa roughness of natural unpolished enamel at baseline following exposure in dietary acid for 10, 20, 30, 40, 50 and 60 minutes. *=P< 0.05, **P<0.01, ***=P<0.001, ****=P<0.0001 vs baseline.

	Baseline	10	20	30	40	50	60
Mean (SD) Sa roughness (µm) n=5	0.268 (0.11)	0.150 (0.062) **	0.125 (0.033) ***	0.146 (0.067) **	0.117 (0.028) ****	0.120 (0.053) ****	0.117 (0.032) ****

Figure 21 shows the representative images selected from the centre of one sample taken at baseline and after 10 and 60 minutes of erosion. At baseline the heavily textured surface was identified and there was evidence of areas of exposed enamel prisms with perikymata and pits visible. After 10 minutes erosion there appears to be an increase in the number of exposed prisms and more defined perikymata, however, after 60 minutes erosion there appears to be a generalised flattening and the

image is similar in appearance to the baseline image. The images showed similarities at all erosion times therefore baseline, 10 minutes and 60 minutes were considered representative.

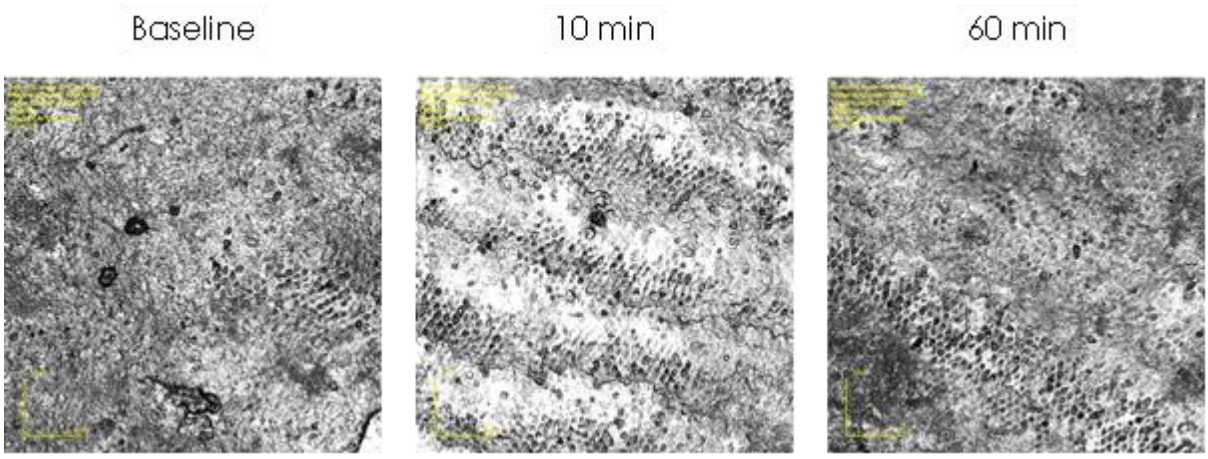


Figure 21: Study 1. Qualitative images of a selected natural unpolished enamel taken at baseline, after 10 minutes of erosion and after 60 minutes of erosion.

2.4 Study 2

2.4.1 Methods

2.4.1.1 Sample preparation

Ten teeth were sectioned as previously outlined in section 2.3.1.1 to provide a buccal, lingual, distal and mesial surface and then randomly allocated to either the natural unpolished or polished groups in order to produce a total of 40 enamel samples (n=20 natural unpolished, n=20 polished). The natural unpolished enamel samples were prepared as previously described in section 2.3.1.1. The polished sample groups were submerged in cold cure acrylic using a customised mould tray and polished flat following previously published protocols (Mistry et al. 2015). The samples were inserted into the polishing machine's automated polishing head (Meta-Serv Vector LC Power Head, Buehler, Lake Bluff, Illinois, USA) using platform ring spacers and secured using a customised jig. The samples were then polished using a series of Silica Carbide Grits (Versocit, Struers A/S, Copenhagen, Denmark) starting with grit size 80 for 3 seconds repeated until the enamel was exposed, followed by size 180 for 6 seconds, 600 for 15 seconds, 1200 for 20 seconds, 2500 for 30 seconds and finally 4000 for 45 seconds under copious water irrigation. To expose the enamel, samples were positioned individually. The size 80 grit was positioned on the rotation plate of the polishing machine, the enamel sample was positioned and secured on the upper plate using customized jigs, the feet of the polishing machine were engaged to emit a force of 10 N on the centre of the samples, the water was switched on, the rotation plate was set to 300 rpm and switched on for 3 seconds. The sample was then inspected and the process repeated as necessary until the enamel was exposed. Following exposure of all samples, four samples at a time were positioned (as previously described) and the process was continued for the entire grit and time sequence. Thus, optically flat enamel samples were prepared with an approximate flatness tolerance of 0.4 µm (Austin et al. 2011). The polishing machine and grit sequence are shown in Figure 22. Both groups of samples were inserted into an ultrasonic bath (Nusonics GP-70, T310) set at 60 Hz for 15 minutes. For the polished samples, PVC tape was applied over the enamel

to create a window of exposed enamel in the centre (approximately 1mm) and a reference area either side, each of equal proportion ($\frac{1}{3}/\frac{1}{3}/\frac{1}{3}$) as shown in Figure 23.

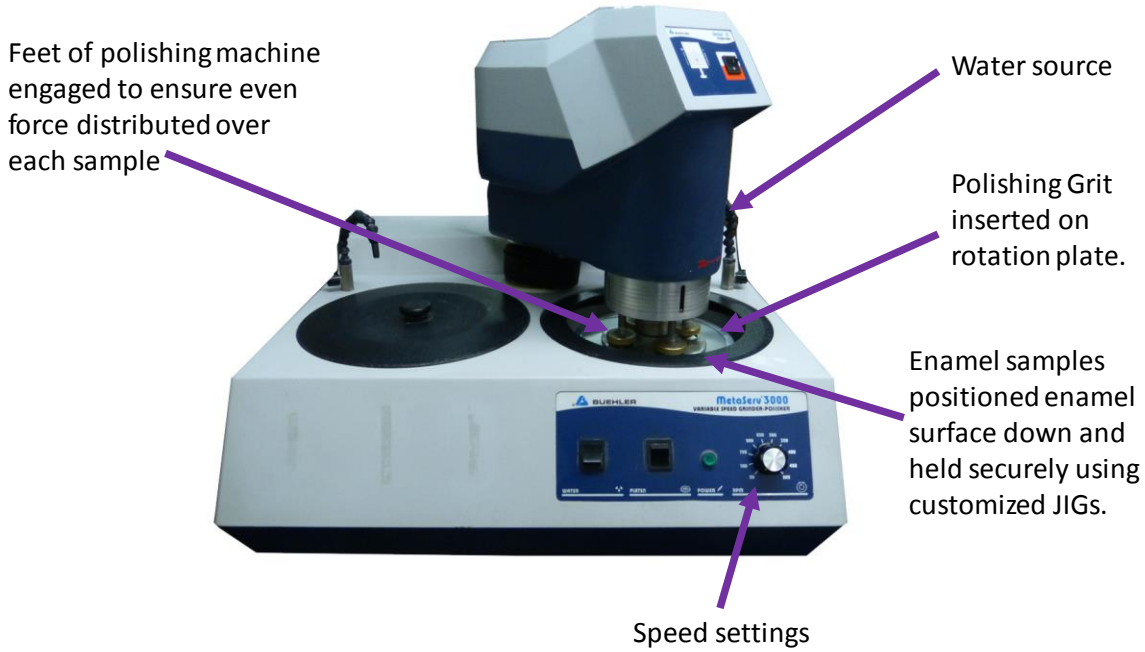


Figure 22: Polishing machine used to prepare optically flat samples.

Table 9: Table showing the protocol used to prepare polished enamel samples.

Silica Carbide Grit Size	Time (s)
80	3 (repeated until enamel exposed)
180	6
600	15
1200	20
2500	30
4000	45

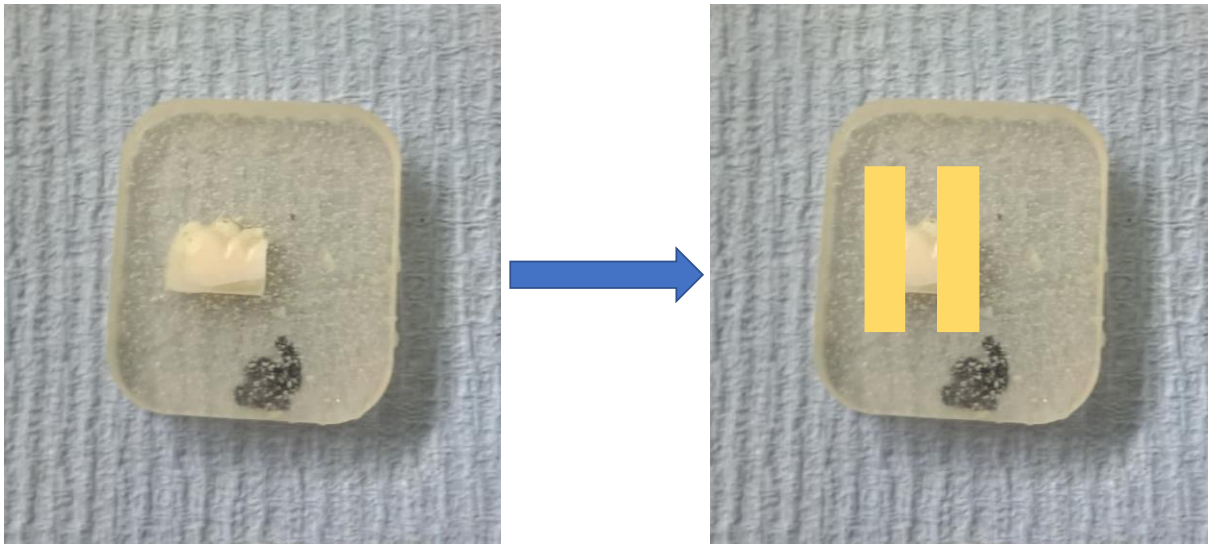


Figure 23: Demonstration of application of PVC tape applied to polished enamel sample to create central window of exposed enamel.

2.4.1.2 Erosion regime

A five-cycle erosion regime using 0.3 % critic acid was used based upon previously published protocols (Mistry et al.2015; O'Toole et al. 2015). Using the previously published protocol for erosion allowed comparison and validation of microhardness and step height measurements, although future studies would utilise commercial products which could eventually be implemented *in vivo.* To prepare the citric acid, the pH meter was calibrated using pH 4 and 7 buffer solutions which was described in section 2.3.1.2 and 1.5 g of citric acid powder was weighed using an electronic analytical scale (Mettler Toledo, XS105 Dual Range Analytical Balance) and dissolved in 500 mL of deionised water in a clean volume flask using a magnetic stirrer. The initial pH was recorded and sodium hydroxide crystals added gradually until the stabilised pH at 3.2. Samples were immersed in batches of 10 into 100 mL of the citric acid (10 mL per sample) under constant agitation at 62 rpm for 10 minutes, then removed and rinsed with deionised water, this was repeated a total of five times as shown in Figure 24.

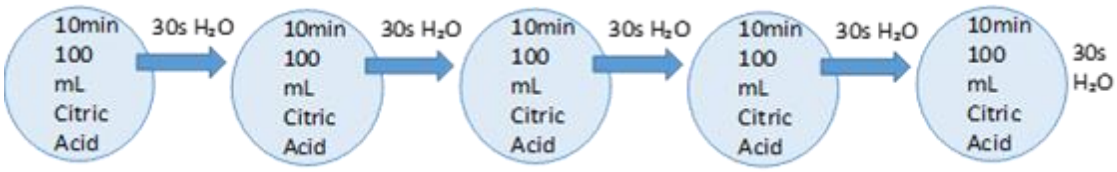


Figure 24: Five-cycle erosion regime used in Study 2.

2.4.1.3 Microhardness and image acquisition

Microhardness testing, using a Knoop Indenter (Duramin-5, Struers Ltd, Rotherham, UK), was carried out at baseline and after erosion for the unpolished and polished enamel samples shown in Figure 25.

Figure 25: Knoop Microhardness Indenter (Duramin-5, Struers Ltd, Rotherham, UK).

Each sample was placed on the indenter's platform and using a 40 X magnification, the focus was adjusted manually to visualise the selected surface. Knoop indentations were made using a force of

981.2 mN and press time of 10 seconds, the sample was moved at least 0.1 mm and another indentation made using the same setting and repeated resulting in a total of 3 indentations. By visually identifying the external borders of the resulting indentations, the Duramin software automatically generated the Knoop Hardness Number using the formula in Equation 3. The accuracy of the tester was 39.33 KHN as measured using a 600 KHN calibrated transfer standard block (Staatliches Materialsprufungsamt Nordrhein-Westfalen, Dortmund, Germany) (Austin et al. 2016).

$$KHN = \frac{F}{C_P \, L^2}$$

Equation 3: The American Society for the Testing of Materials (ASTM) formula for calculation of the Knoop Hardness Number (KHN). Where F is the load (kg) and L is the length of the long diagonal (mm). C_P is the indenter constant.

The three recordings were then averaged for each polished sample before and after erosion. Hardness change was calculated by subtracting mean hardness value after erosion from the baseline. Any samples outside a baseline microhardness of 270 KHN – 400 KHN were discounted and other samples sourced to achieve the desired sample size. This baseline range was based upon previous extensive validation studies (Austin 2011). However, only some baseline measurements were possible for natural unpolished enamel.

Step height measurements were not possible for the natural unpolished enamel samples as the PVC dislodged during the erosion cycling. However, the polished samples were scanned with a white light profilometer (Xyris 4000, TaiCaan, Southampton, UK) with Stages software (TaiCaan, Southampton, UK) after erosion shown in Figure 26. The white light profilometer had a spot size of 7 µm, angular tolerance 38 - 40˚ and reported vertical resolution of 10 nm (TaiCaan 2016) and the stage movements were directed by the motion control software which was accessible as a desktop application upon a

customised Windows 7 computer. The optical principles of the system followed the confocal principle whereby the light was emitted onto the measured surface through a confocal aperture as described in section 2.3.1.3 The light source was centred in the middle of the eroded zone for each sample and scan settings set to ensure both reference areas and the eroded zone were scanned equally (approximately 3 mm by 3mm) at a 10 µm scanning interval. The z axis was adjusted to achieve focus, whereupon the software flashed red and further adjustments were made to achieve a focal range of 350 µm (Austin 2011). Once optimum focus was achieved the sample was scanned in a raster pattern with the stage moving in the x & y axes. The resulting scan images were analysed using BODDIES analysis software (TaiCaan, Southampton, UK). Three representative profiles were extracted and the vertical step height measured from the midpoint of the eroded zone to each reference area in accordance to ISO 5436 standards as shown in Figure 27. This provided a total of six step height measurements per sample which were then averaged.

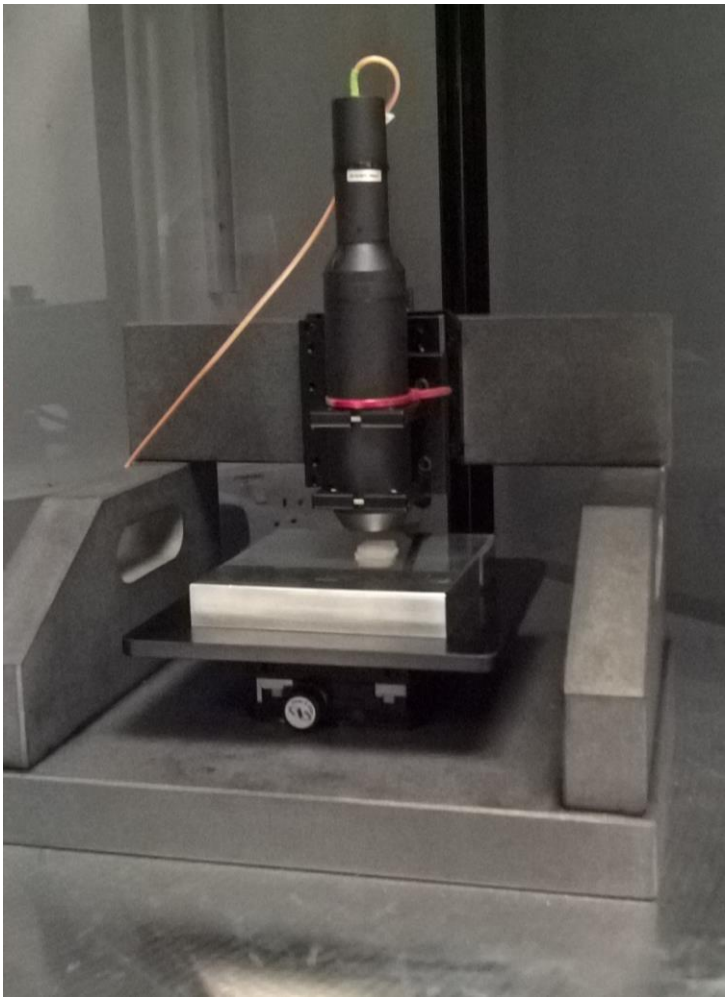


Figure 26: White Light Confocal Profilometer (Xyris 4000 TaiCaan, Southampton, UK).

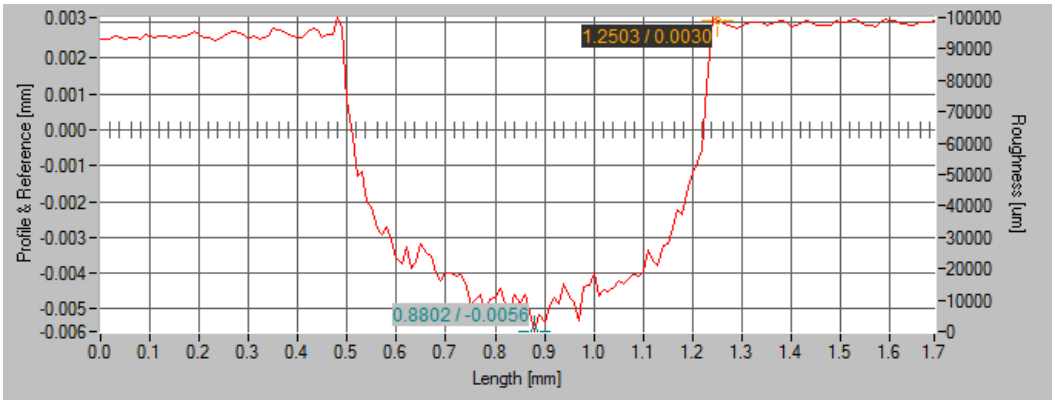


Figure 27: Schematic representation of step height measurement. The step height was measured from the centre of the eroded zone to a reference zone both sides, and the two readings averaged.

2.4.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA). Histogram plots and Shapiro Wilk studies were used to determine normality. The data were normally distributed therefore a T test was used to compare microhardness at baseline vs after erosion with significance set at $p<0.05$.

2.4.2 Results

From the 20 natural unpolished enamel samples, microhardness measurements could only be obtained from 10 (50 %) samples which had a mean (SD) of 295.3 (123.3) KHN at baseline with no data possible following erosion.

For the polished enamel samples, data was recorded from all samples and showed a statistically significant reduction in mean (SD) microhardness from 322.7 (28.4) KHN to 178.3 (50.1) KHN following erosion ($P< 0.001$). Resulting in a mean (SD) microhardness change of 144.4 (52.5) KHN shown in Figure 28.

The mean (SD) step height for the polished enamel samples was 6.17 (2.01) µm.

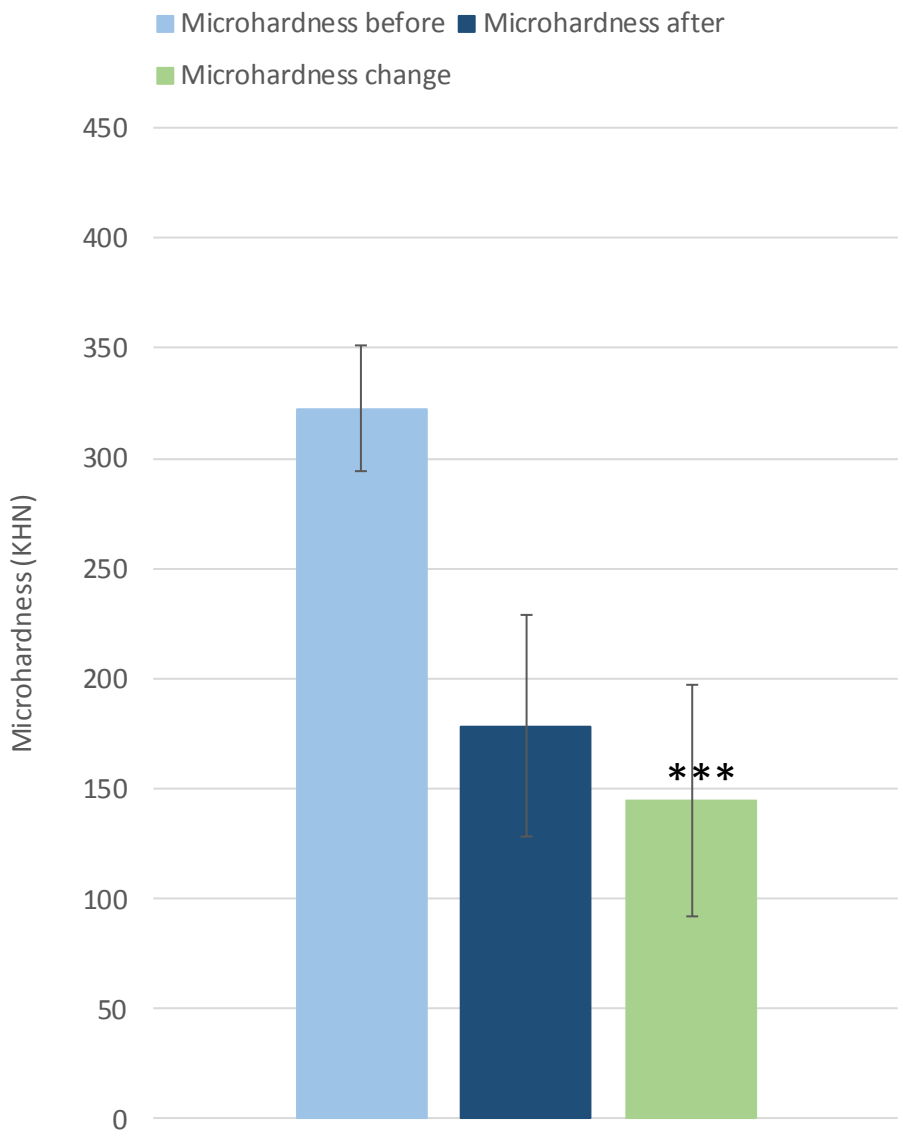


Figure 28: Study 2. Mean (SD) microhardness of polished enamel before and after erosion in 0.3% citric acid for polished enamel samples. *** = P< 0.001

2.5 Study 3

2.5.1 Methods

2.5.1.1 Sample preparation

A total of 30 natural unpolished and 30 polished enamel samples were prepared from buccal sections as previously described in sections 2.3.1.1 and 2.4.1.1 Following preparation, the samples were randomly sub divided in 3 groups (n=10 natural unpolished, n=10 polished per group). In Group 1, impression compound (Kerr™ Corporation, USA) was applied over the enamel to create the window of exposed enamel described in section 2.4.1.1. In Group 2 PVC tape (INCOM Manufacturing Group Ltd, Canada) was used to create the same effect, and in Group 3 nail varnish (The Color Institute, Markwins® International, USA) was used. Following erosion cycling, using the method described below, both the impression compound and PVC tape were removed manually and the nail varnish was gently removed using acetone. The assessment of residue left behind from the barriers was assessed visually using the CLM described in section 2.3.1.3

2.5.1.2 Erosion regime

Samples were immersed in batches of 10 in 100 mL of orange juice under constant agitation at 62 rpm for 15 minutes, then rinsed in distilled water by spraying the samples from a water bottle held approximately 5 cm from the samples for 30 seconds, completing one cycle. This was repeated twice as described in Figure 29.

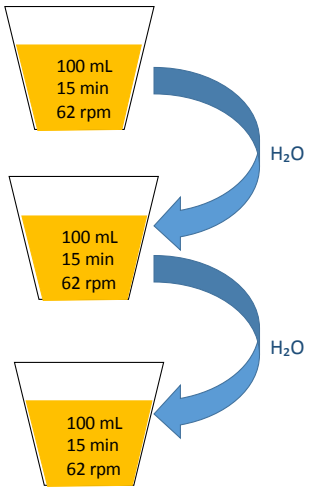


Figure 29: Three-cycle erosion regime used in Study 3.

2.5.1.3 Microhardness and image acquisition

Microhardness was tested on polished samples at baseline and after erosion as previously described in section 2.4.1.3

Surface roughness was measured using a red laser confocal profilometer on the natural unpolished and polished enamel samples. The red laser profilometer measurement system consisted of a red laser displacement meter (LT-9010M, Keyence Corporation, Japan), a motion controlled stage (Xyris 2000, TaiCaan, Southampton, UK) and measurement software (Stages TaiCaan, Southampton, UK) as shown in Figure 30.

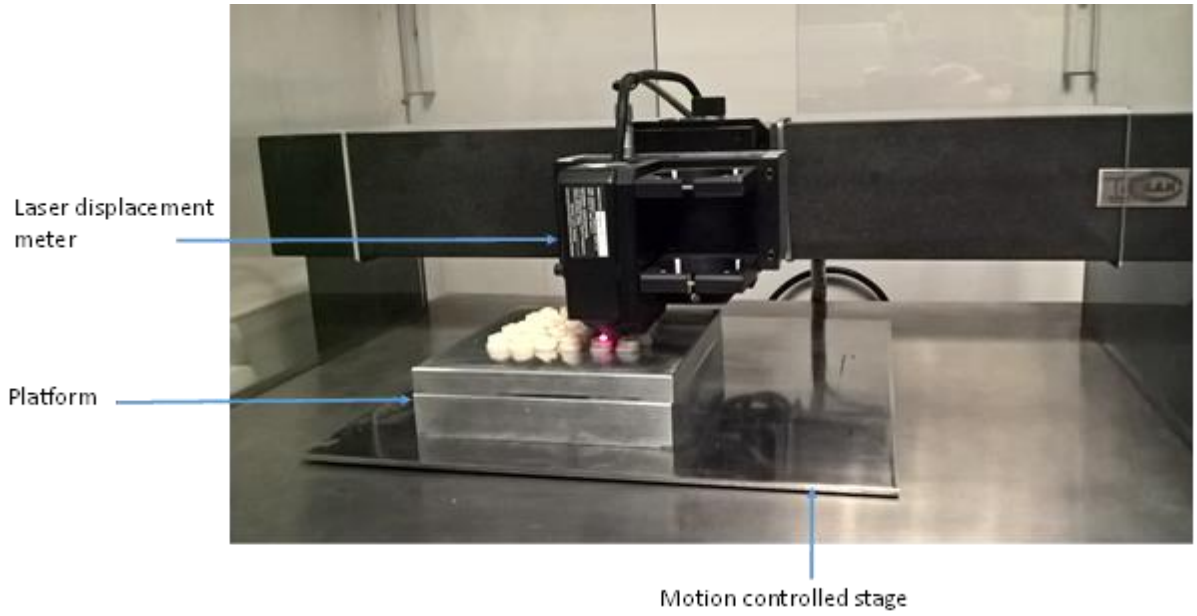


Figure 30: Red laser confocal profilometer (Xyris 2000, TaiCaan, Southampton, UK).

The samples were imaged, after erosion, by conveniently selecting five areas (each 0.04 mm²) in the eroded zone of each samples and five areas (each 0.04 mm²) selected from one reference area, with a green horizontal guide lined to indicate optimum focus as shown below in line Figure 31.

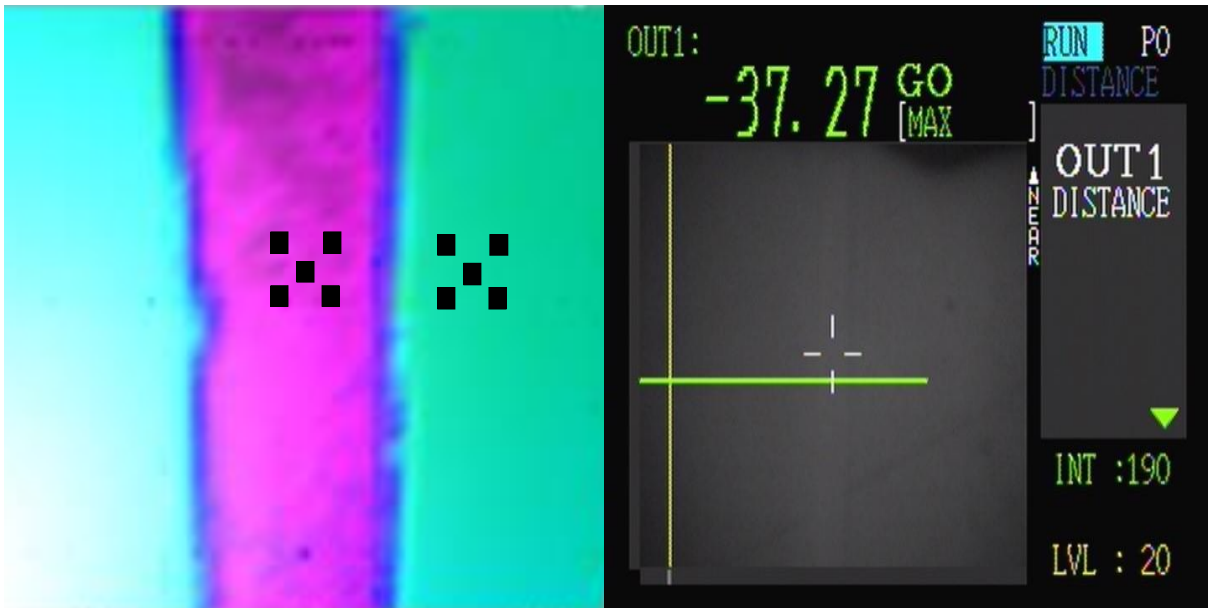


Figure 31: Representative image of how five areas were selected in the eroded area and one reference area to measure surface roughness and the video image demonstrating the green horizontal indicator line.

The stage movements were directed by the motion control software which was accessible as a desktop application upon a customised Windows 7 computer. The red laser spot size was 2 µm, vertical resolution 100 nm and flatness tolerance of 20-25˚ (TaiCaan 2016). A scanning interval of 4 µm was selected. The optical principles of the system followed the confocal principle. Once optimum focus was achieved the sample was scanned in a raster pattern with the stage moving in the x & y axes. The scans were analysed using MountainsMap (DigitalSurf, France). They were levelled using the least squared method, a 25 µm Gaussian filter applied to filter out the waviness and form data leaving the roughness data from which Sa roughness was extracted. Finally, the assessment of residue left behind from the barriers was assessed by imaging the samples using the CLM described in section 2.3.1.3 for qualitative assessment.

2.5.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA). Histogram plots and Shapiro Wilk tests were used to determine normality. The data were normally distributed therefore T tests were used to compare the Sa roughness of baseline vs after erosion with significance set at $p<0.05$.

2.5.2 Results

For natural unpolished enamel samples, seven recordings were obtained from the impression compound with Sa roughness decreasing from a mean (SD) Sa of 0.85 (0.31) µm measured from the reference area, to 0.78 (0.28) µm measured from the eroded zone. There were three recordings each for the PVC tape and nail varnish possible due to measurement drop out. The Sa from the PVC tape samples increased from 0.70 (0.17) µm to 0.74 (0.12) µm, whilst the nail varnish samples decreased in Sa from 0.82 (0.27) µm and 0.80 (0.17) µm shown in Table 10. The data that was not possible to be analysed was caused by drop out over the sloped reference areas. The qualitative images taken with the CLM revealed residue from both nail varnish and impression compound.

Table 10: Study 3. Mean (SD) Sa roughness before vs after erosion of natural unpolished enamel samples (n=30) comparing reference barriers. ^{ns}=P>0.05.

Barrier Type	Mean (SD) Sa roughness reference (µm)	Mean (SD) Sa roughness eroded (µm)
Impression compound	0.85 (0.31)	0.78 (0.28) ^{ns}
PVC tape	0.70 (0.17)	0.74 (0.12) ^{ns}
Nail varnish	0.82 (0.27)	0.80 (0.17) ^{ns}

For polished enamel samples Sa roughness significantly increased after erosion regardless of the reference barrier used. Impression compound samples significantly increased in surface roughness from 0.18 (0.09) µm to 0.33 (0.13) µm (p<0.01), the PVC tape samples significantly increased in surface roughness from 0.28 (0.17) µm to 0.37 (0.10) µm (p<0.05) and nail varnish samples significantly increased in surface roughness from 0.45 (0.11) µm to 0.64 (0.45) µm (p<0.01).

For impression compound samples, there was a significant reduction in KHN of 342.47 (25.75) KHN before to 217.46 (53.45) KHN after erosion (P< 0.001), for the PVC tape samples, microhardness reduction after erosion from 319.05 (22.56) KHN to 176.70 (48.83) KHN (p <0.001) and for the nail varnish samples hardness reduced from 228.41 (54.00) KHN to 158.63 (21.98) KHN p <0.001) as shown in Table 11.

Table 11: Study 3. Mean (SD) Sa roughness and microhardness before vs after erosion of polished enamel samples comparing differing reference barriers ***=P<0.001

Barrier Type	Mean (SD) Sa roughness before (µm)	Mean (SD) Sa roughness after (µm)	Mean Microhardness before (KHN)	Mean Microhardness after (KHN)	Mean Microhardness Change (KHN)
Impression compound	0.18 (0.09)	0.33 (0.13) ***	342.47 (25.75)	217.46 (53.45) ***	125.02 (60.99)
PVC Tape	0.28 (0.17)	0.37 (0.10) ***	319.05 (22.56)	176.70 (48.83) ***	142.35 (43.92)
Nail varnish	0.45 (0.11)	0.64 (0.45) ***	228.41 (54.00)	158.63 (21.98) ***	69.77 (63.28)

2.6 Study 4

2.6.1 Methods

2.6.1.1 Sample preparation

Following qualitative observations of acrylic sample surface contamination on natural unpolished enamel samples in Study 3, the embedding material was changed to bisacryl composite for all future sample preparation. Buccal sections of enamel were fully embedded in bisacryl composite (Protemp4, 3M, ESPE, UK), using the original mould trays. They were polished as described in Study 2 section 2.4.1.1 above to produce 20 samples. Ten samples were randomly allocated for further preparation using diamond polishing paste (DP Stick P 1 µm grain size, Struers, Roper Technologies, Inc, USA). A polishing cloth (MD/DP Floc, Struers, Roper Technologies, Inc, USA.) was inserted onto the rotation plate of the polishing machine. The paste was applied to the polishing cloth and the samples positioned using the arm of the polishing machine and polished for 60 seconds at 300 rpm. All samples were rinsed with distilled water and immersed in the ultrasonic bath for 15 minutes. PVC tape was used to create a window of exposed enamel and two reference areas as previously described in section 2.4.1.1.

2.6.1.2 Erosion regime

The same three-cycle erosion regime from section 2.5.1.2 was used.

2.6.1.3 Microhardness and image acquisition

Microhardness was tested on the samples at baseline and after erosion as previously described in section 2.4.1.3.

Surface roughness measurements were recorded using the red laser confocal profilometer. Five areas (each 0.04 mm^2) were selected in the centre of the enamel samples at baseline and after erosion. They were analysed for Sa roughness change as previously described in 2.5.1.3.

2.6.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA). Histogram plots and Shapiro Wilk studies were used to determine normality. Data were normally distributed and T tests were used to compare baseline vs. after erosion and Sa roughness change between the two groups and microhardness change between the two groups with significance set at $p<0.05$.

2.6.2 Results

There were statistically significant differences between the baseline mean (SD) Sa roughness values for the polishing paste group 0.11 (0.06) µm and the no paste group 0.04 (0.01) µm ($p<0.01$). Following erosion, the mean (SD) Sa roughness significantly increased from 0.11 (0.06) µm to 0.20 (0.08) µm ($p< 0.05$) for the paste group resulting in a mean (SD) roughness change of 0.10 (0.06) µm. Mean (SD) Sa roughness significantly increased following erosion from 0.04 (0.01) µm to 0.22 (0.04) µm ($p< 0.01$) for the no paste group resulting in a mean (SD) roughness change of 0.18 (0.03) µm. Both are shown in Figure 32. However, there was no statistical significant difference in roughness change between the paste group and no paste group ($p>0.05$).

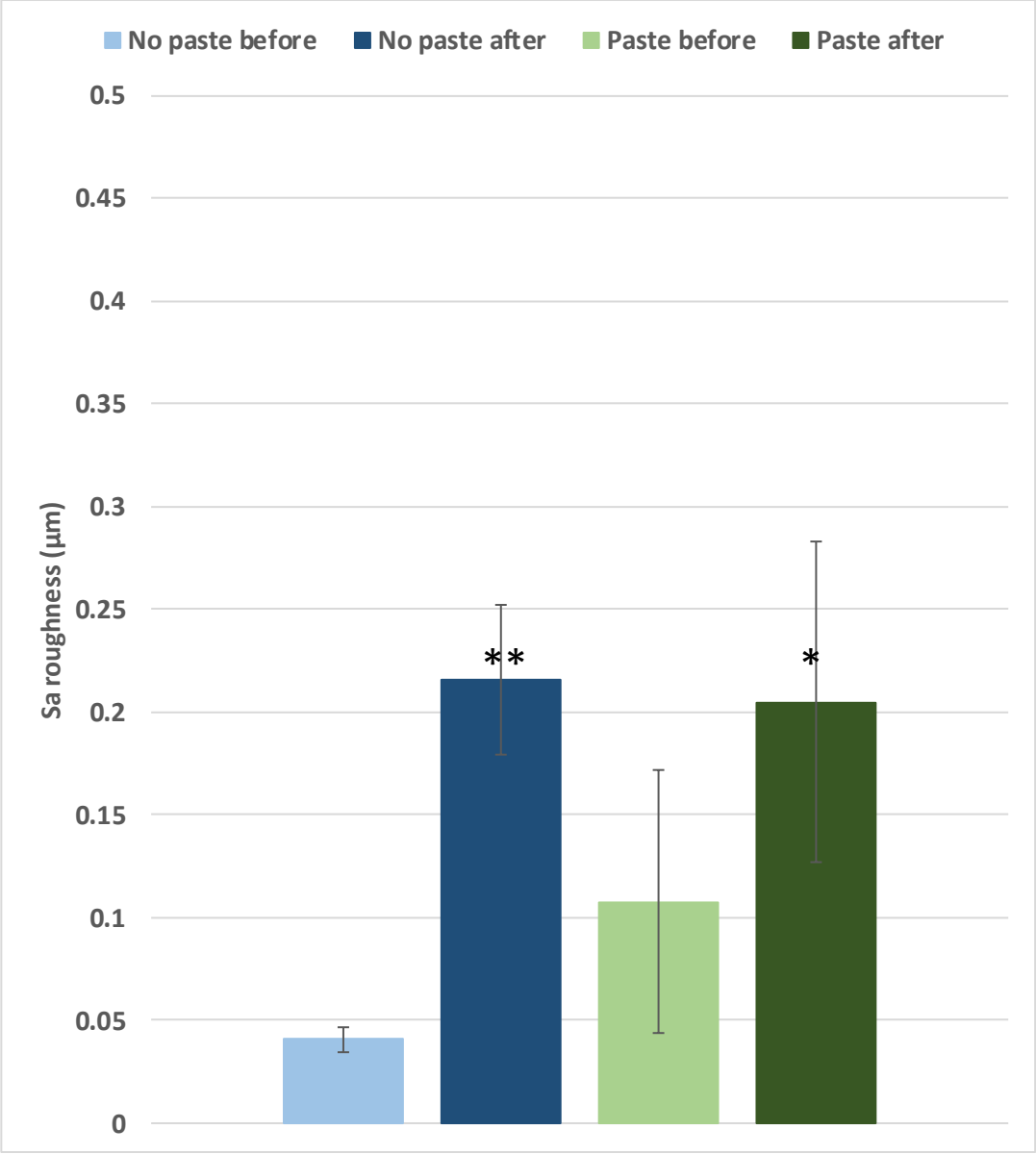


Figure 32: Study 4. Mean (SD) Sa roughness before and after erosion samples prepared with polishing paste vs no paste. *=P<0.05, **=P<0.01

Mean (SD) microhardness at baseline was 317.8 (17.5) KHN for the paste group and 308.0 (18.2) KHN for the no paste group, these were not statistically significant (p>0.05). Following erosion, the paste group significantly decreased in microhardness to 185.2 (27.6) KHN (p<0.001) and the no paste group significantly decreased in microhardness to 119.5 (23.2) KHN (p<0.001). The resulting hardness change

of 132.6 (27.6) KHN for the paste group and 119.5 (23.2) KHN for the no paste group were not statistically different (p>0.05) and are shown in Figure 33.

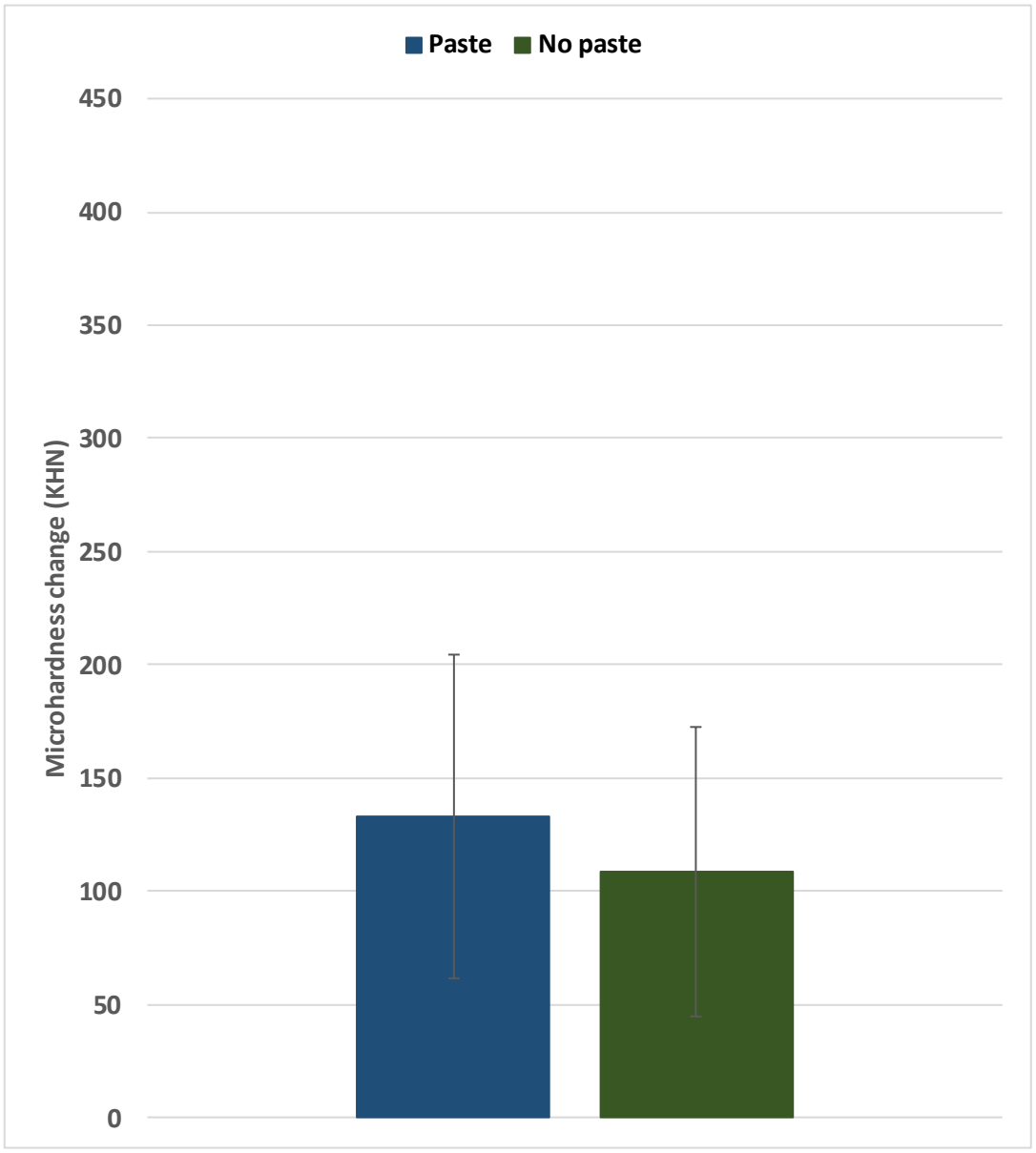


Figure 33: Study 4. Mean (SD). Microhardness change of samples prepared with polishing paste vs no paste. There was no statistical difference between the groups. P>0.05

2.7 Study 5

2.7.1 Methods

2.7.1.1 Sample preparation

Buccal sections of natural unpolished enamel were embedded in bisacryl composite (Protemp4, 3M, ESPE, UK) ensuring the outer surface was left untouched. They were cleaned using a soft toothbrush and non-fluoridated toothpaste (Kingfisher, Norwich, UK) and the smear layer removed with ethanol to mimic the cleaning procedures which could be implemented clinically. The samples were then randomly allocated into two equal groups.

2.7.1.2 Erosion regime

The same three-cycle erosion regime (described in Study 3 section 2.5.1.2) was used to compare Group 1 (Sainsbury's basic orange juice - an orange juice made from concentrate) and Group 2 (Sainsbury's basic orange juice drink -a pre-mixed diluting drink with citric acid added by the manufacturer). Group 1 had a pH of 3.9 and TA of 110.5 mmol OH/L and Group 2 had a pH of 3.2 and TA of 41.3 mmol OH/L.

2.7.1.3 Image acquisition

Sa roughness change was measured using the red laser confocal profilometer as described in section 2.6.1.3.

2.7.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA). Histogram plots and Shapiro Wilk studies were used to determine normality. The data were not normally distributed therefore Mann-Whitney Rank Sum tests were used with significance set at $p<0.05$ and data expressed as Median (IQR).

2.7.2 Results

Figure 34 shows Group 1 (the orange juice) had a median (IQR) Sa roughness 0.40 (0.45) µm at baseline and 0.36 (0.17) µm after erosion and this difference was not statistically significant ($p>0.05$). Group 2 had a median (IQR) Sa roughness of 0.62 (0.28) µm at baseline and 0.39 (0.06) µm after erosion ($p<0.01$).

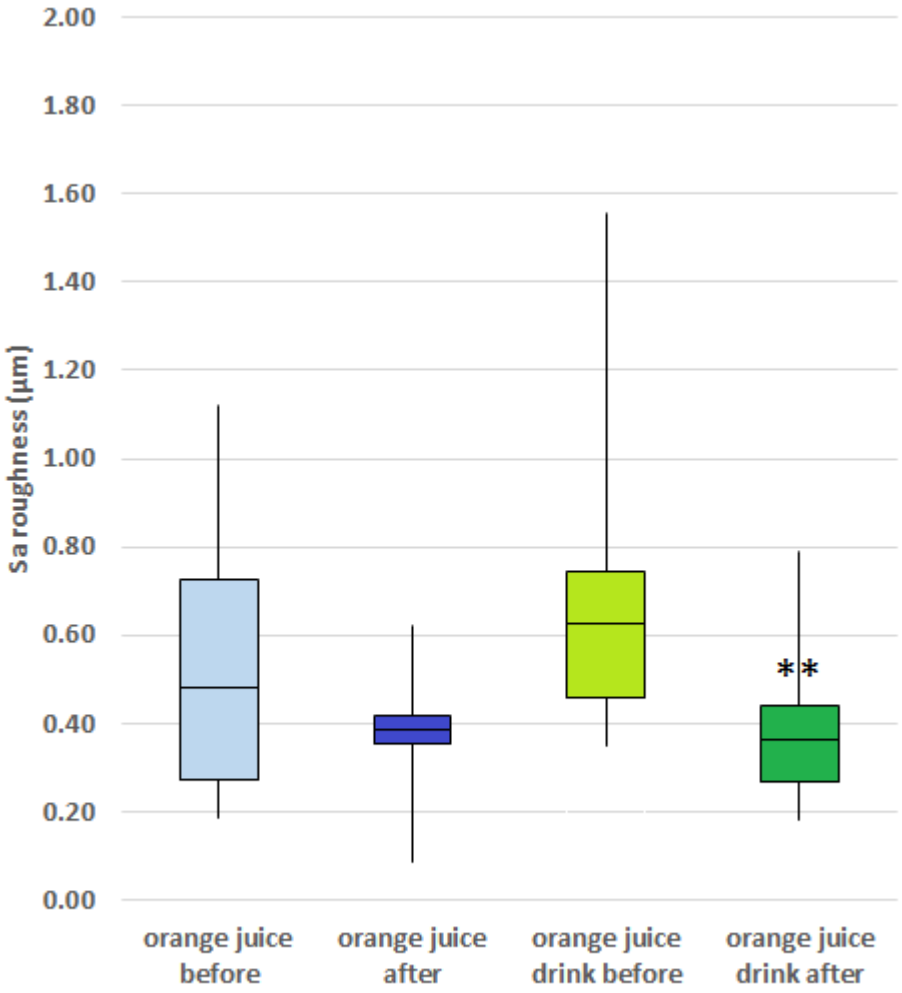


Figure 34: Study 5. Boxplot demonstrating Sa roughness before and after 45 minutes immersion of natural unpolished enamel samples in orange juice vs orange juice drink. **=P<0.01

2.8 Study 6

2.8.1 Methods

2.8.1.1 Sample Preparation

Ten natural unpolished enamel samples were prepared as previously described in Study 5 section 2.7.1.1 and baseline impressions recorded of each enamel section using polyvinyl siloxane impression material type-3 low-consistency (Extrude light bodied, Kerr, UK) and repeated after erosion. The impressions were used as negative replicas.

2.8.1.2 Erosion regimes

The three-cycle erosion regime used the orange juice drink and was described in section 2.7.1.2.

2.8.1.3 Image acquisition

To measure surface roughness of the replicas of the natural unpolished enamel samples, using the red laser confocal profilometer, five areas (each 0.04mm²) were selected in the centre of the sample and the replica impression and imaged at a 4 µm scanning intervals at baseline and after completion of erosion as previously described in section 2.6.1.3. Sa roughness was extracted from all scans using the methods previously described in 2.5.1.3.

2.8.1.4 Statistical analysis

Statistical analysis was conducted using SPSS version 22 (IBM, USA) Histogram plots and Shapiro Wilk studies were used to determine normality. The data were not normally distributed therefore Mann-Whitney Rank Sum tests were used with significance set at $p<0.05$.

2.8.2 Results

The median (IQR) Sa roughness at baseline of the natural unpolished enamel was 0.62 (0.27) µm and from the impressions was 0.57 (0.19) µm which were not statistically different ($P>0.05$) as shown in

Figure 35. The median Sa roughness (IQR) after erosion of the natural unpolished enamel was 0.38 (0.06) µm and the impressions was 0.44 (0.29) µm which were not statistically different (P>0.05), also shown in Figure 35.

The median (IQR) Sa roughness of the natural unpolished enamel significantly decreased from 0.62 (0.27) µm to 0.38 (0.06) µm after erosion (P<0.01). However, measuring from the impression the Sa median (IQR) of the replica impressions decreased from 0.57 (0.19) µm to 0.44 (0.29) µm but it was not statistically significant (P>0.05).

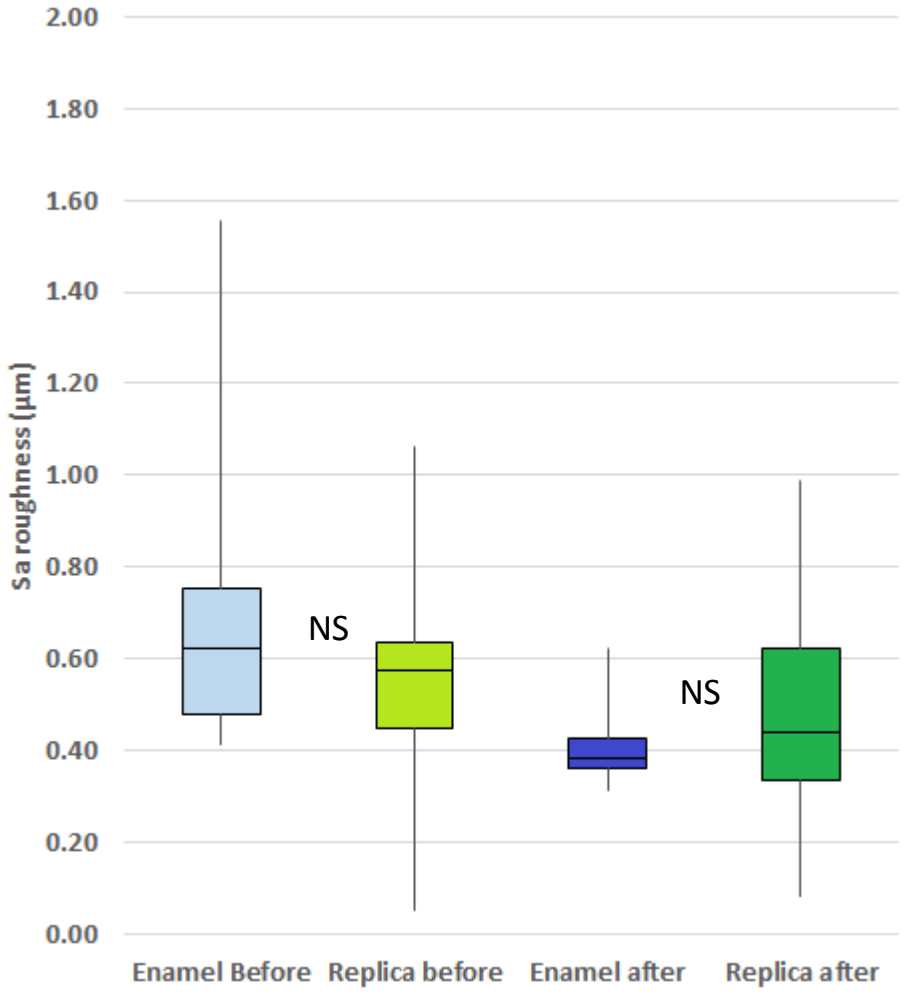


Figure 35: Study 6. Boxplot demonstrating Sa roughness measuring natural unpolished enamel vs impression replica.

2.9 Discussion

Overall this chapter helped to develop the methods to quantify changes in surface roughness of natural unpolished and polished enamel following erosion by dietary acid to be used in the rest of the thesis. It was observed that surface roughness of natural unpolished enamel significantly decreased following erosion whereas it increased for polished enamel. This is supported by findings from other studies (Mann et al. 2014; Austin et al. 2016; Hara et al. 2016; Ranjitkar et al. 2016; Mullan et al. 2017b). It was further observed that neither microhardness measurements nor the use of reference barriers were feasible for natural unpolished enamel samples. Both the microhardness change and step height loss measured in this chapter were consistent with other work which used the same study protocols (Mistry et al. 2015).

The red laser profilometer used in Studies 2 to 6 was newly purchased and to initiate the development of a method to quantify surface roughness changes of natural unpolished natural enamel a traceable gold standard CLM was used for the first study. Traceability is the basis of which measurements can be considered accurate (Leach 2014). Traceability is defined as "*property of a measurement result relating the result to a stated metrological reference through an unbroken chain of calibrations of a measuring system or comparisons, each contributing to the stated measurement uncertainty*" (ISO 2004).

The decision to measure five scan areas was based on convenience and following advice from dimensional metrologists at the NPL and TaiCaan Technologies. In addition, measuring natural unpolished enamel with both the CLM and red laser confocal profilometer identified higher measurement error on the periphery (naturally sloped areas) of the natural unpolished enamel compared to centre of the samples/ apex of curvature. This is supported by observations from other authors (Ranjitkar et al. 2016). The sloped areas result in increased drop out as they pushed the limits of angular tolerance of the red laser confocal profilometer, therefore subsequent studies (after Study 3) compared Sa roughness measurements taken from five areas selected in the centre of the samples

before and after erosion. A different choice of Gaussian filter was used for the red laser confocal profilometer compared to the CLM study, and was subsequently implemented for the remainder of the thesis. The red laser confocal profilometer was lower in resolution than CLM and therefore less detail was observed rendering the previous filter process unsuitable as it removed too much detail. A new Gaussian filter of 25 µm was chosen based upon the estimation that this filter was five times the diameter of an enamel prism and following collaboration from industrial dimensional metrologists at the NPL and TaiCaan Technologies. Overall, this suggested that the red light laser although less accurate than the CLM was sufficient for roughness measurements.

Using natural unpolished enamel is more clinically representative, but it is understood that natural enamel will have more variation than polished enamel where the outer layer has been removed. It has been identified that the concentrations of calcium, fluoride and phosphorus decrease towards the DEJ (Nakagaki et al. 1987; Weatherell et al. 1974). The outer enamel of unerupted and erupted teeth significantly differs in fluoride concentration, which could affect research outcomes when studying erosion (Mizuno et al. 1990). Therefore, only erupted molars were used throughout this this thesis. However, following collection the teeth were anonymised and no details regarding age, diet, location and fluoride history were recorded. In an attempt to minimise variation only buccal surfaces of molars were used, with the exception of Study 2 (Carvalho and Lussi 2015; Mistry et al. 2015).

There is some suggestion that natural unpolished enamel becomes smoother following erosion and this more closely mimics the clinical pattern of erosive tooth wear. Teeth which are diagnosed with erosive wear are often described as having a 'smooth and shiny appearance' (Bartlett 2005). Furthermore, an *in vivo* study by Whitehead et al. (1997) investigated surface roughness changes in a small longitudinal observational study by measuring acrylic replicas of participants' teeth. They reported a reduction in surface roughness over 3 months using 2D measurements from a contacting profilometer with a 5 µm head. In theory, the diameter of the stylus was the same as an enamel prism. Further work was needed to utilise the high-resolution equipment, which is now available, to identify

micro-structural enamel changes. Studies which have been published after this initial work was completed provide further support for its novel findings (Arnold et al. 2015; Hara et al. 2016; Mullan et al. 2017b). Arnold et al. (2015) identified non-significant reductions in surface roughness of natural unpolished enamel following erosion with HCL. Hara et al. (2016) identified a statistically significant reduction in surface roughness of natural unpolished enamel samples following erosion. The surface roughness of natural unpolished enamel is more variable than polished enamel with baseline values differing throughout the studies. Moreover, there were differences in trends of surface roughness changes in Study 3. The impression compound and nail varnish samples showed a decrease in roughness, however, the PVC samples demonstrated an increase in roughness. As only three measurements were possible for this group it was not possible to draw conclusions from this, but it does demonstrate that there is increased variability when measuring surface roughness of natural unpolished enamel compared to polished enamel. The measurement technique for this study was based upon previous studies measuring step height where the reference areas underneath barriers such as tape and nail varnish are scanned post operatively only (Mistry et al 2015). However, to determine if the placement and removal the reference barriers directly affected the enamel surface; the difference between baseline measurements and measurements following removal of the barrier should have perhaps been considered. Overall, there would have little difference in the outcome for the thesis as it was deemed for other reasons (namely presence of residue identified using qualitative assessment and the increased measurement drop out measuring sloped areas) that the reference barriers were not appropriate. In all future studies Sa roughness measurements would be recorded at baseline and repeated following erosion without the use of reference barriers.

The red laser confocal profilometer was a higher resolution non-contact profilometer with a spot size of 4 µm and deemed more appropriate for fine detail surface roughness measurements compared with the pre-existing white light profilometer in the department (which had a spot size of 7 µm). However, the white light profilometer had been validated through extensive research for step height

measurement, for which it was used for in this chapter (Austin 2011; Mistry et al. 2015). Therefore, both were used to compare data and to assess the output from the new red laser profilometer.

Microhardness is recommended for use on highly polished surfaces (Attin & Wegehaupt 2014). However, Torres et al. (2010) investigated % microhardness change on natural unpolished enamel surfaces following increasing immersion times. They stated that they used the flattest part of the surface. In an attempt to find the flattest available surfaces mesial and distal sections of enamel were included for Study 2, as these regions are naturally flatter than bulbous buccal molar enamel. However, there were inconsistencies in attempts to measure all four sides equally and perhaps paradoxically the apex of the curvature provided the better area to examine. Overall there was no consistency to the measurements as the indents were rarely clear and certainly not consistent. Therefore, it was concluded that microhardness is not suitable for measuring effects of erosion on natural unpolished enamel.

In the Literature Review the importance of standardised polishing protocols was described (Carvalho & Lussi 2015). Polishing regimes in erosion studies vary amongst different authors. In one *in vitro* study investigating preventative effects of stannous fluoride and chitosan products on erosion and abrasion, Carvalho and Lussi (2014), polished their samples using a series of carbide grits from 500 to 4000 and completed this with the use of two diamond pastes. For an *in situ* study examining the initial stages of enamel erosion, Parkinson et al. (2010), polished bovine enamel samples using a series of silica carbide gits 400, 1200, 2400 and 4000 completing the process with a diamond polishing paste and cloth. However, not all others use polishing paste for their samples. In an *in vitro* investigating the effects of sodium fluoride formulations of erosion and abrasion study Austin et al. (2011) used a series of grits from 500 to 4000 but no polishing pastes and achieved a flatness tolerance of 0.4µm. Therefore, this questions the need for polishing paste. It was identified in this chapter that when polishing paste was used it resulted in significantly higher baseline surface roughness. This could suggest that residue was left behind despite ultrasonication. Moreover, as the diamond paste is abrasive in nature this could

be causing the increase in surface roughness, similar to that identified with toothbrush abrasion (Mullan et al. 2017a). There were no statistical difference in baseline microhardness or microhardness change between the two groups. However, where surface roughness is being used as an indicator of erosive wear diamond polishing paste was not appropriate as part of the polishing regime and polishing paste was not used for the remainder of this thesis.

Two types of orange juice were used in this chapter. The first (Sainsbury's orange juice from concentrate) was chosen as it had been used in previously published protocols (Hooper et al. 2007; Austin et al. 2017). However, it was not possible to identify changes in surface roughness of natural unpolished enamel with the red laser confocal profilometer following immersion in this orange juice. Therefore, a potentially more erosive beverage (Sainsbury's orange juice drink, which had a lower pH and citric acid added by the manufacturer) was trialled. Immersion in this product resulted in detectable changes in surface roughness of natural unpolished enamel with the red laser confocal profilometer and this product was used for the remainder of the thesis. Different measures can be used to identify the erosive potential of beverages including pH and titratable acidity (TA) (Laurance-Young et al. 2011). With the two beverages used in this chapter, the pH of the orange juice drink was more acidic but the TA less acidic implying that pH has more influence over erosive potential. This supports other studies which prefer assessing buffering capacity, which measures H^+ at a certain pH value, to TA which measures total available H^+ over a range of pH values (Lussi et al. 2012). Furthermore, the orange juice drink contained citric acid added by the manufacturer which may have contributed to it exerting a more erosive effect. The red laser confocal profilometer had a lower resolution than the CLM and therefore, required a more vigorous erosion regime to successfully identify changes in surface roughness.

A replica technique was trialled to investigate if the method for quantifying surface roughness was suitable *in vivo*. The impression material used for the replica was chosen for its dimensional stability, and had been validated in a previously (Rodriguez & Bartlett 2011). Previously, Hjortsjö et al. (2012)

identified a correlation between surface topography of enamel and replicas of enamel at a profile level measuring Pa (profile average) of both the enamel surfaces and positive acrylic replicas. However, whether the level of replication was adequate for fine roughness quantification such as Sa was unclear. This chapter identified a close correlation between measuring the negative impression replicas versus the enamel at baseline and after erosion. However, it was unable to detect roughness changes when measuring the negative replicas. Perhaps this false negative measuring the replicas was due to reduction in accuracy and precision measuring the smoother surface as previous studies have identified that replica techniques are less accurate when quantifying smoother surfaces (Goodall et al. 2015). Another factor could be the differences in accuracy and precision when measuring a concave versus a convex surface. Accuracy and precision are both reduced for curved surfaces compared to flat surfaces (Hewlett et al. 1992). The same principle applies to concave versus convex surfaces. Therefore, positive replicas cast in acrylic could overcome this issue and should be used for future replica studies. Overall, the replica technique was considered successful due to the close correlation between the values achieved measuring the replica versus the original surface, which is in agreement with other studies (Goodall et al. 2015).

2.10 Conclusions

The overall conclusions from this Chapter were that surface roughness of natural unpolished enamel became significantly smoother following dietary erosion whereas, surface roughness of polished enamel becomes significantly rougher. Microhardness measurements and the use of reference barriers were not suitable for natural unpolished enamel samples. Surface roughness measurements should be taken from the apex of natural unpolished enamel samples to reduce measurement drop out.

Chapter 3 Validation of the measurement protocol using the red laser confocal profilometer

3.1 Introduction

To fully interpret surface roughness measurements, it is important that the measurement capability of the equipment and analysis techniques are understood. Techniques which are widely established in engineering and manufacturing processes can assist with this. Traceability was described in the previous chapter, section 2.9. Traceable measurement equipment is not commonly found except in national measurement institutes such as the National Physical Laboratory, so it is not feasible to demand that all equipment used in biological studies, such as those measuring tooth wear, must be traceable (Leach 2000). When a measuring device is not traceable a series of three measurements can be carried out to understand the measurement error involved; namely to identify inherent noise, accuracy and precision (which is broken down further into repeatability and reproducibility) (Smith et al. 2007).

An observed measurement consists of the true measurement and the measurement error (Smith et al. 2007). The inherent error (background noise) of a measuring apparatus is the combination of internal noise (instability in the instrument electronics), environmental noise (temperature, floor vibrations) and the noise of the x and y drive units in the measurement when scanning along the z axis. The resolution of measuring equipment is the smallest detectable movement of the instrument and is dependent on the diameter of the light source spot size or diameter of the stylus depending if non-contact or contact devices are being used (Giusca & Leach 2013; Hocken et al. 2005; Durakbasa et al. 2011). These influence the capability of the measurement equipment and are inherent. However, whilst they cannot be reduced they can be measured or estimated which in turn will help to validate a measured value. For example, if a measured value is within the realms of the recorded noise one could not be certain if the measurement is the actual surface or the inherent noise.

To fully validate measurement equipment, accuracy and precision are also to be considered. Precision and accuracy are two terms which are often interchangeable in everyday life, however, in metrology they have two distinct meanings. Accuracy is the closeness of agreement of a measurement to a known value and precision is the closeness in agreement of repeated measurements of an unknown value, which can be expressed quantitatively as repeatability and reproducibility (Leach 2014). Repeatability is the closeness in agreement of a series of measurements where there has been no change to conditions and is considered an assessment of the measurement equipment. Reproducibility is the closeness in agreement of a series of measurements but with a change to conditions between each measurement, and is considered an assessment of the operator. In both cases the mean and standard deviations are calculated for the series of measurements and the standard deviations are used to express the 'precision'. These can be compared using statistical methods including F tests and confidence intervals (Austin & Elliott 2014; Borror et al. 1997).

As well as investigating the precision and accuracy of measuring equipment there are also ISO standards dedicated to help identify the accuracy of the analysis software. These are referred to as soft gauges and are algorithms which can be analysed with the users' analysis software and the numerical results compared with the 'true value' (Leach et al. 2006).

3.2 Aims

- To identify the inherent measurement error of the red laser confocal profilometer (Xyris 2000, Taicaan, Southampton UK) using a profilometer a glass lithium-aluminosilicate 1/20th lambda single surface optical flat (Zerodur, Schott Edmunds Industrial Optics).
- To assess the accuracy of the red laser confocal profilometer using a 2.97 µm Ra roughness standard (Brown&Sharpe, TESA, Switzerland).
- To investigate the precision of the red laser profilometer measuring surface roughness of flattened and naturally curved enamel samples.

- To assess the accuracy of surface metrology software (MountainsMap DigitalSurf, France) at measuring surface roughness.

3.3 Null hypothesis

- The red laser confocal profilometer and software are not accurate or precise systems.

3.4 Study 1

3.4.1 Methods

3.4.1.1 Sample preparation

A glass lithium-aluminosilicate 1/20th lambda single surface optical flat and 2" diameter (Zerodur, Schott Edmunds Industrial Optics) was scanned.

3.4.1.2 Image acquisition and analysis

To identify the baseline measurement noise of the measurement system, following good practice guidelines (Giusca et al. 2012) a 0.04 mm^2 area (200 microns by 200 microns) was conveniently selected from the centre of the optical flat and scanned with the red laser confocal profilometer at a scanning interval of 4 µm. The red laser confocal profilometer was described in Chapter 2 section 2.5.1.3. The resulting scan was analysed using surface metrology software (MountainsMap DigitalSurf, France). The scan was levelled using the least squared method, a 25 µm Gaussian filter applied to filter out the waviness and form data leaving the roughness data in order to quantify Sz roughness (Giusca et al. 2012). An example of the filtering process is show in Figure 36.

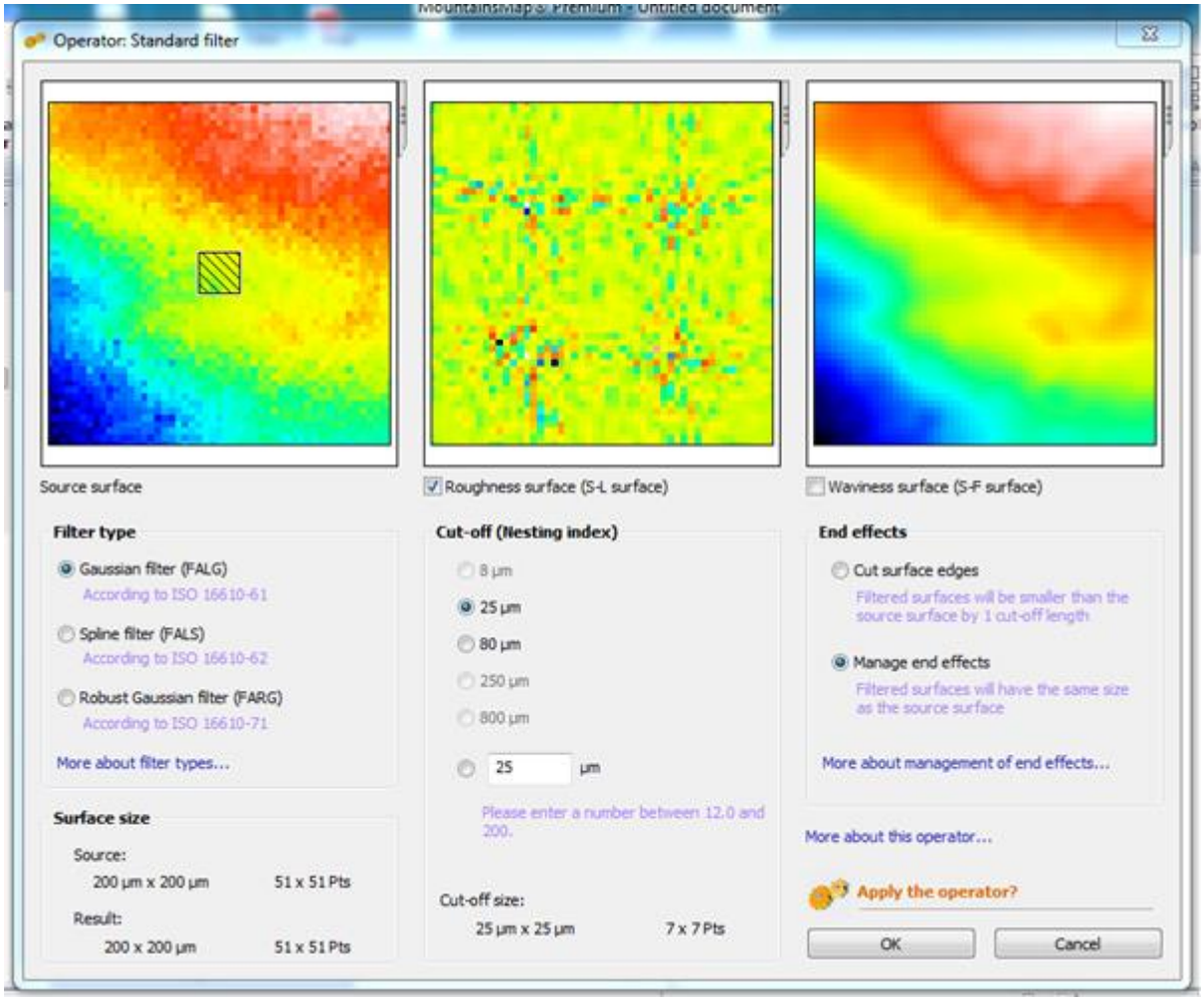


Figure 36: Example of the filtering process. A 25 µm was applied to supress the longer wavelengths and extract the roughness data (which is the centre image).

3.4.2 Result

The inherent error of the measurement was measured as 5.79 nm.

3.5 Study 2

3.5.1 Methods

3.5.1.1 Image acquisition and analysis

A 1 mm² area was conveniently selected on a 2.97 µm roughness standard (Brown&Sharpe, TESA, Switzerland) and scanned with the red laser confocal profilometer using a scanning interval of 10 µm.

The scans were analysed using BODDIES (TaiCaan, Southampton, UK) analysis software in accordance with ISO 13565, a 0.8 mm Gaussian filter applied and Ra roughness extrapolated. Readings were recorded from 5 profiles and averaged.

3.5.2 Results

The mean (SD) Ra of the roughness standard as measured by Ra was 3.11 (0.12) µm resulting in an accuracy of 0.14 µm or 95.3 %.

3.6 Study 3

3.6.1 Methods

3.6.1.1 Sample preparation

One natural unpolished and one polished enamel sample were embedded in bisacryl composite and prepared as previously described in sections 2.6.1.1 and 2.7.1.1.

3.6.1.2 Image acquisition and analysis

To assess repeatability five areas (each 0.0 4mm²) were conveniently selected from the central zone of one natural and one polished enamel sample as shown in Figure 37. They were scanned using the scan settings described in section 2.5.1.3. Each area was then scanned 30 times without any change to conditions, at a scanning interval of 4 µm using the red laser confocal profilometer. The resulting 300 scans were analysed for Sa roughness as previously described in section 2.5.1.3. For each scan area the mean and standard deviation of the 30 measurements were calculated. The standard deviations for each area were used to express precision and were compared (Leach 2014).

Figure 37: Representation of the five areas selected in the centre of the unpolished and polished enamel samples to assess precision.

To assess reproducibility five areas were conveniently selected for one natural unpolished enamel sample and one polished enamel sample and scanned immediately. The sample was then removed from the stage for a minimum of 1.5 hours and left, following which the samples were replaced and the five areas per samples were re-reselected for scanning. This was repeated a total of 30 times. The resulting 300 scans were analysed for Sa roughness as previously described in section 2.5.1.3. (Leach 2014).

3.6.1.3 Statistical analysis

For the precision of measurement, the repeatability and reproducibility were expressed by calculating the variability (SD) of the 30 measurements (µm) for each roughness area. Histogram plots were used to determine if the repeatability and reproducibility data were normally distributed, data sets which were not normally distributed were log transformed if positively skewed. F tests were used to compare

the standard deviations amongst each repeatability and reproducibility group with P<0.05 considered statistically significant (Austin & Elliott 2014).

3.6.2 Results

Table 12 shows the results of the precision (SD in µm) of measurement of enamel 3D (Sa) surface roughness measurements for 30 repeatability and reproducibility measurements of the five scan areas on the natural unpolished enamel sample and the polished enamel sample. For repeatability of natural unpolished enamel the standard deviations differed over the different areas measured. The smallest standard deviation was 0.007 µm and the largest 0.023 µm with statistical differences between area 1 and 3, 4, 5 (P<0.001) and area 2 and 3, 5 (P<0.001). The reproducibility (SD) data for natural unpolished enamel revealed that there was up to 15 times more variability, the smallest standard deviation was 0.097 µm and the largest standard deviation was 0.130 µm, however there were no statistically significant differences between the standard deviations from any of the five areas (p>0.05).

For repeatability of polished enamel the standard deviations differed over the areas measured. The smallest standard deviation was 0.001 µm and the largest standard deviation was 0.005 µm, with statistical differences between areas 1 and 5 (P<0.001), 2 and 4 (P<0.05), and 3 and 4 (P<0.01). The reproducibility (SD) data for polished enamel revealed that there was up to 20 times more variability in the data, the lowest standard deviation 0.021 µm and the largest standard deviation was 0.025 µm, however there were no statistically significant differences between any of the 5 measurement areas (p>0.05).

Table 12: Precision (SD in µm) of enamel 3D (Sa) surface roughness measurements of 30 repeatability and reproducibility measurements of five scan areas from the centre of natural unpolished enamel and polished enamel.

		Area of enamel section				
		1	**2**	**3**	**4**	**5**
Natural unpolished enamel	**Repeatability (SD in µm)**	0.023	0.023	0.007	0.013	0.011
	Reproducibility (SD in µm)	0.130	0.097	0.108	0.101	0.102
Polished Enamel	**Repeatability (SD in µm)**	0.004	0.005	0.001	0.001	0.001
	Reproducibility (SD in µm)	0.021	0.025	0.024	0.020	0.025

3.7 Study 4

3.7.1 Methods

3.7.1.1 Sample preparation

Three soft gauges of polished surfaces, which were surface files with a "true" value were sourced from the UK National Measurement Institute, the National Physical Laboratory (NPL Teddington, UK). These were downloaded following completion of a registration form.

3.7.1.2 Image Analysis

The three surface files were analysed by applying a 0.8 mm Gaussian filter, in accordance with ISO 13565. The results were compared to the 'true value' (NPL 2004).

3.7.2 Results

The 'true values' of the Ra roughness of the soft gauges were 0.063 µm, 0.079 µm and 0.231 µm, whilst the values using the test software (MountainsMap) were 0.061 µm, 0.091 µm and 0.227 µm respectively. There were no statistically significant differences between these values as shown in Table 13.

Table 13: True values for the three soft gauges analysed versus the values extracted following analysis using MountainsMap analysis software.

Reference profile	True value of Ra (µm)	Test value (µm)
1	0.063	0.061
2	0.079	0.091
3	0.231	0.227

3.8 Discussion

This series of four studies was carried out to identify the measurement capability of the red laser confocal profilometer and to validate the method developed to characterise enamel in Chapter 2. These studies were based upon previously published protocols to identify measurement capabilities (Giusca et al. 2012; Leach 2001; Smith et al. 2007; Menditto et al. 2007; Mullan et al. 2017b). The studies identified the inherent error, accuracy and precision of the red laser confocal profilometer measuring natural unpolished and polished enamel and the accuracy of the analysis software. The relationship of these terminologies is shown in Figure 38.

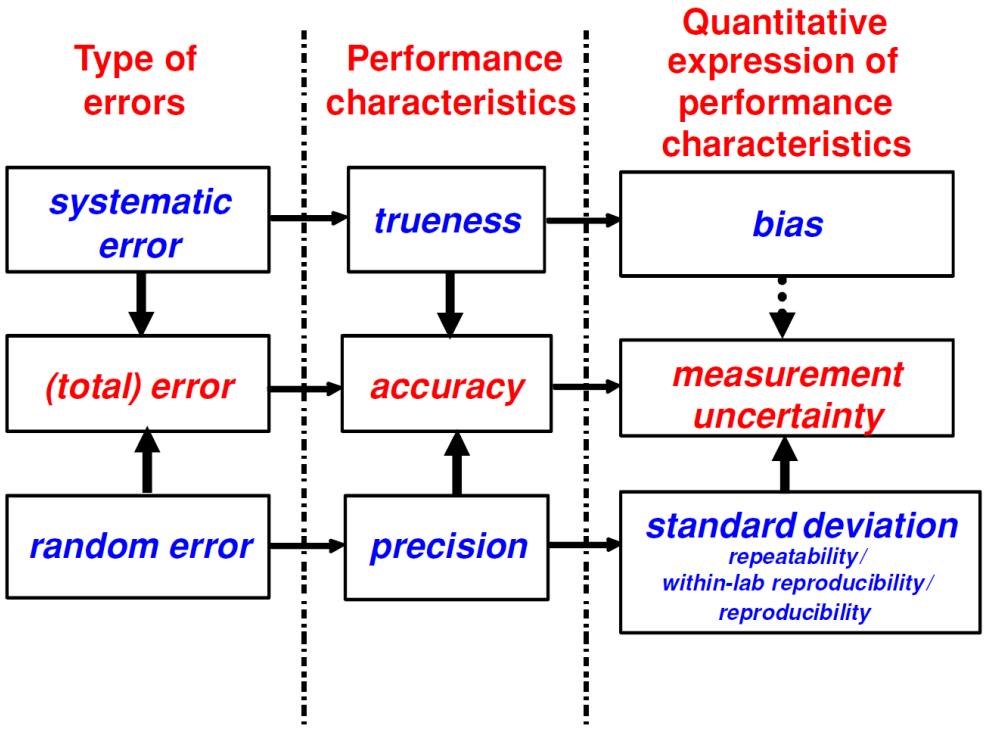


Figure 38: Relationships between type of error, qualitative performance characteristics and their quantitative expression (Menditto et al. 2007).

Measuring the inherent error of the red confocal profilometer was essential in understanding the measurement capability (Menditto et al. 2007). Every measured value is combined from a true value and measurement error. The value of a measured surface must be greater than the inherent error to be considered valid. Therefore, in the case of the red light confocal profilometer the roughness values for enamel surfaces must be greater than 5.97 nm, which they were and confirms the suitability of using the instrument.

Accuracy is the closeness in agreement of a measured value to a known value, which in this case was the 2.97 µm Ra ISO roughness standard (Menditto et al. 2007). It provides an assessment of the measurement capability when measuring an ideal surface. However, one can expect that the level of accuracy will alter depending upon the surface measured (Hewlett et al. 1992). The agreement between the true value and the measured value was 95.3 %, demonstrating an accurate measurement device and rejecting the null hypothesis. This percentage value of accuracy has similarities to that reported by other authors using non-contact profilometry to measure step height (Hara et al. 2016; Paepegaey et al. 2013). The accuracy of analysis software is also important for identifying measurement capabilities. This was assessed by comparing Ra (2D) measurement using soft gauges, which is another established method in metrology (NPL 2004). The concept is similar to assessing the accuracy of the measurement equipment with a roughness standard. However, in the case of soft gauges the measurement has already been completed under traceable conditions creating a surface file with a 'true value'. Analysis of this file (following the predetermined ISO standards) and comparing the value achieved with the 'true value' identifies the accuracy of the analysis software. The overall accuracy of 99.6 % for the test software (MountainsMap, DigitalSurf, France) rejects the null hypothesis.

Precision is broken down into repeatability and reproducibility which are expressed as standard deviations. However, further statistical analysis of the equal variance of the measurements can also be used to investigate the relationship between the standard deviations further (Borror et al. 1997;

Leach 2014; Smith et al. 2007). Repeatability was assessed by measuring five distinct areas of natural unpolished and polished enamel 30 times consecutively without any changes to conditions and subsequently comparing the standard deviations for these series of measurements. The five areas were chosen in the centre of the samples based upon work described in the previous chapter. These areas were selected to represent the position on the tooth where the enamel prisms were most likely to meet the surface perpendicular and optimise the measurement conditions (Nanci & Ten Cate 2008). The decision to measure 30 times was based upon previous work (Austin & Elliott 2014) and discussed with a statistician. There were statistically significant differences between the five areas for both natural unpolished and polished samples which suggested random rather than systematic measurement errors. Moreover, the measurement capability was lower for curved natural surfaces, which was anticipated (Hewlett et al. 1992). The increased error for the natural unpolished enamel samples implied that the most reliable measurement zone on natural unpolished enamel was at the apex of the curvature and so this site was used for all future studies and will be investigated further in the next Chapter. This was the central zone of the sample, whereas in contrast the slope areas resulted in increased drop out as they push the limits of angular tolerance as experienced in Chapter 2.

Accuracy and precision vary upon the type and shape of surface measured. Hewlett et al. (1992) measured the Z height of a grade-25 chrome-steel precision sphere at 1˚ increments and these were compared with the true values to assess accuracy. Precision was calculated by dividing the square of the precision by the Mean Squared Error for each slope angle. They identified a steady decrease in accuracy and precision with increase in surface angle steadily from 0˚ to 90˚. These principles were used in another study, Pintado et al. (1997) investigated the accuracy and precision of two contact profilometers (R and T) measuring curved surfaces (a grade 5 precision ball bearing with a diameter of 4.0000 mm ± 0.0002 mm). They identified the mean accuracy and precision for the surface angles from 0˚ (horizontal) to 60˚ degrees were R= 4 μm, T= 5 and R = 3, T = 3, respectively concluding that

for angles less than 60˚ the accuracy was better than 7 µm and the precision better than 5 µm regardless of stylus. They used this to validate tooth wear measurements calculating volume loss versus depth (Pintado et al. 1997). Schlueter et al. (2005) identified decreased precision measuring step height loss on natural unpolished enamel samples compared with flattened samples from standard deviations of 2.2 µm to 3.9 µm. Optical profilomoters are also known to be most accurate when they are measuring flat surfaces positioned perpendicular to the incident light beam the most light is returned to produce high resolution scan images. Whereas in contrast, steeply curved or sharp edges will result in distortion of light thereby increasing the margin of error and explaining the larger SDs seen for natural unpolished enamel in this chapter (Durakbasa et al. 2011). Furthermore, accuracy and precision of optical profilometers will vary dependent upon the specular and diffuse nature of the surface to be measured (Nostell et al. 1999). Therefore, it was important to assess precision using the natural unpolished and polished enamel samples that would be used throughout this thesis.

Reproducibility was assessed by scanning five areas per sample 30 times removing and replacing the samples between each scan and waiting a minimum 1.5 hours. This assessed the operator's ability to relocate the same areas. Reproducibility was lower in precision than repeatability (larger standard deviations), however there were no statistical differences among the five measurements for both natural unpolished and polished enamel. This is of clinical importance, as the use of fixed reference markers is not advocated for *in vivo* studies as they easily dislodge (Sundaram et al. 2007). The lack of statistical differences among the measurements also suggests a sufficient level of precision thereby rejecting the null hypothesis.

Different filters are appropriate for different structures and surfaces, often this is guided by ISO standards. A specific term for the accuracy component of the type of validation in this Chapter is Gauge Capability Studies and because of this Studies 2 and 4 were governed by the ISO standard to use 2D roughness and 0.8 mm filters which are appropriate for the stainless-steel surfaces measured. However, the parameter of choice for quantifying surface roughness of human enamel was 3D (Sa)

roughness. 3D (Sa) is calculated from an overall surface making it more representative of a complex structure such as enamel, and is more appropriate. Unfortunately, human enamel does not have an ISO standard recommending filters for its analysis. Therefore, the choice of filter for this thesis was chosen based upon the structure of enamel, the resolution of the device for measuring and professional advice from industrial dimensional metrologists at NPL and TaiCaan Technologies as described in Chapter 2.

3.9 Conclusions

Overall, accuracy and precision experiments identified a reliable measurement system and technique to measure surface roughness of natural and polished enamel samples and the null hypothesis was rejected.

Chapter 4 Comparing Sa roughness over different locations of natural unpolished and polished enamel

4.1 Introduction

Measurements from the centre, or apex of curvature, of curved surfaces increase accuracy and precision of surface texture measurement of natural unpolished enamel samples (Ranjitkar et al. 2016). However, it was unknown if Sa roughness measurements from the centre of the samples were representative of Sa roughness of the overall surfaces of natural unpolished enamel and polished enamel. Previous work using qualitative SEM assessment suggested that erosive changes varied over different locations on the surface of natural unpolished enamel. Meurman and Frank (1991) examined qualitative effects of erosive wear on polished and natural unpolished enamel. They identified variations in erosion pattern which they linked to inherent structural variations within natural unpolished enamel and specifically within aprismatic enamel. Other studies which have compared measurements using different tooth sides and tooth types have also identified variations in both polished and natural unpolished surfaces of human teeth (Carvalho & Lussi 2015; Mistry et al. 2015). Polishing regimes have been shown to produce different surface finishes dependent if the tooth substrate is human, bovine or ovine (Field et al. 2014). The angulation of enamel prisms varies over different locations, in the centre of the samples they are mainly perpendicular to the surface. However this differs over the periphery and may influence the overall surface characteristics (Braly et al. 2007). From the previous chapters it was identified that measurement from the centre of natural unpolished enamel samples was more reliable due to there being higher precision and less 'drop out'. However, it has not been established whether the central zone could be considered representative of the overall sample with regards to Sa roughness measurements.

4.2 Aims

- To compare measuring Sa roughness from 5 scan areas located in a single central cluster versus 20 scan areas located over four peripheral clusters (5 scan areas per cluster) for natural and polished enamel.
- To investigate changes in Sa roughness of natural unpolished and polished enamel following erosion with dietary acid.

4.3 Null hypothesis

- Sa roughness is not the same over the surface of enamel even after exposure to a dietary acid.

4.4 Methods

4.4.1 Sample preparation

10 natural unpolished enamel and 10 polished enamel samples were prepared in bisacryl composite as previous described in Chapter 2 sections 2.6.1.1 and 2.7.1.1.

4.4.2 Erosion regimes

The three-cycle erosion regime using the orange juice drink pH 3.2, TA 41.25 mmol OH/L described in Chapter 2 section 2.7.1.2 was used.

4.4.3 Image acquisition

4.4.3.1 Red laser confocal profilometer

To compare Sa roughness over different locations of natural unpolished enamel and polished enamel, and to identify changes in Sa roughness following erosion with dietary acid, Sa roughness was measured at baseline and after erosion in both a single central cluster (0.5 mm wide) and four equally sized peripheral clusters. Each cluster was approximately 1.5 mm apart and conveniently selected to represent the entire surface. Within each cluster, five smaller areas (each 0.04 mm^2) provided the Sa roughness data as shown in Figure 39 below. The five areas within each cluster were methodically selected using the horizontal guideline from the live video link to identify the areas of best intensity

and a scan interval of 4 µm was used. The resulting scans were analysed for Sa roughness as described in Chapter 2 section 2.5.1.3. The Sa roughness from the five scan areas in the single central cluster were averaged to provide the data for the single central cluster and the Sa roughness from the 20 scan areas which made up the four peripheral clusters were averaged to provide the peripheral cluster data.

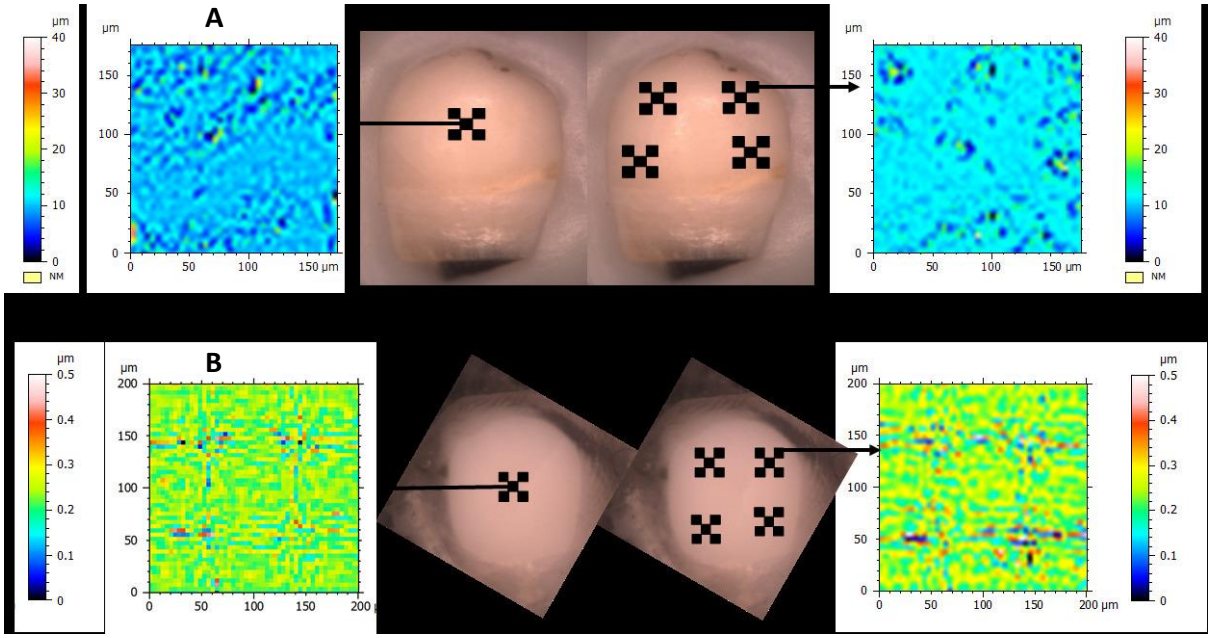


Figure 39: Representative diagrammatic images of a natural unpolished (A) and polished enamel (B) sample with the 5 scan areas for the central cluster and 20 scan areas for the peripheral cluster mapped out separately. A representative baseline scan image for the central cluster.

4.4.3.2 ESEM

One natural unpolished enamel sample and one polished enamel sample were randomly selected for imaging with Environmental Scanning Electron Microscopy (ESEM) (Phenum ProX desktop SEM Phenom-World BV, The Netherlands) before and after erosion. The selected samples were mounted onto specimen holders, cleaned with ethanol and blast dried with an air spray. Each specimen was inserted into the ESEM machine. Five representative areas on the surface were selected similarly to the method used for the surface roughness measurements to provide a single image of the central

cluster and one each of the peripheral clusters. The scan area used for the roughness measurements was 200 X 200 µm, therefore to image an area of similar size, magnification at 1100 X was selected providing a total area of 246 X 246 µm. The ESEM had a reported resolution of ≤ 14 nm. The ESEM emitted a 5Kv electron beam onto the surface of the specimen, and during scanning the secondary electrons released from each point of the scanned surface were detected and used to build up a high-resolution image. Contrast and brightness were adjusted manually to achieve best possible images.

One representative central ESEM scan and one representative peripheral ESEM scan were selected for natural unpolished enamel and polished enamel before and after erosion.

4.4.4 Statistical Analysis

To determine the sample size, a calculation was carried out using G*Power3.1.92 (Heinrich-Heine-University, Dusseldorf). The mean and standard deviations were based on results from previous studies (0.67 (0.13) µm for natural unpolished enamel and mean (SD) of 0.12 (0.02) µm for polished enamel). SPSS (IBM, United States) was used for the statistical analysis, as the data were non-normally distributed. Friedman tests were used with post-hoc multiple comparisons using paired Wilcoxon tests comparing the results for the single central cluster versus the four peripheral clusters and comparing the results before erosion versus after erosion. A Bonferroni correction for multiple comparisons was carried out and significant difference was set at $p<0.008$. The statistical analysis was carried out by a statistician.

4.5 Results

The single central cluster for natural unpolished enamel had a median (IQR) Sa roughness of 1.45 (2.58) µm and the four peripheral clusters had a median (IQR) of 1.32 (4.86) µm before erosion, which reduced to 0.38 (0.35) µm and 0.34 (0.49) µm respectively after erosion ($p<0.0001$). There were no statistical differences between measuring the single central cluster to the four peripheral clusters at baseline ($p>0.008$) and after erosion ($p>0.008$). These are shown in Figure 40.

The Median (IQR) Sa roughness of the polished enamel was 0.04 (0.17) µm in the single central cluster and 0.05 (0.15) µm for the four peripheral clusters before erosion, increasing to 0.27 (0.08) µm and 0.27 (0.08) µm, respectively, after erosion ($p<0.0001$). These are shown in Figure 41. There were no statistical differences between measuring the single central cluster to the four peripheral clusters at baseline ($p>0.008$) or after erosion ($p>0.008$).

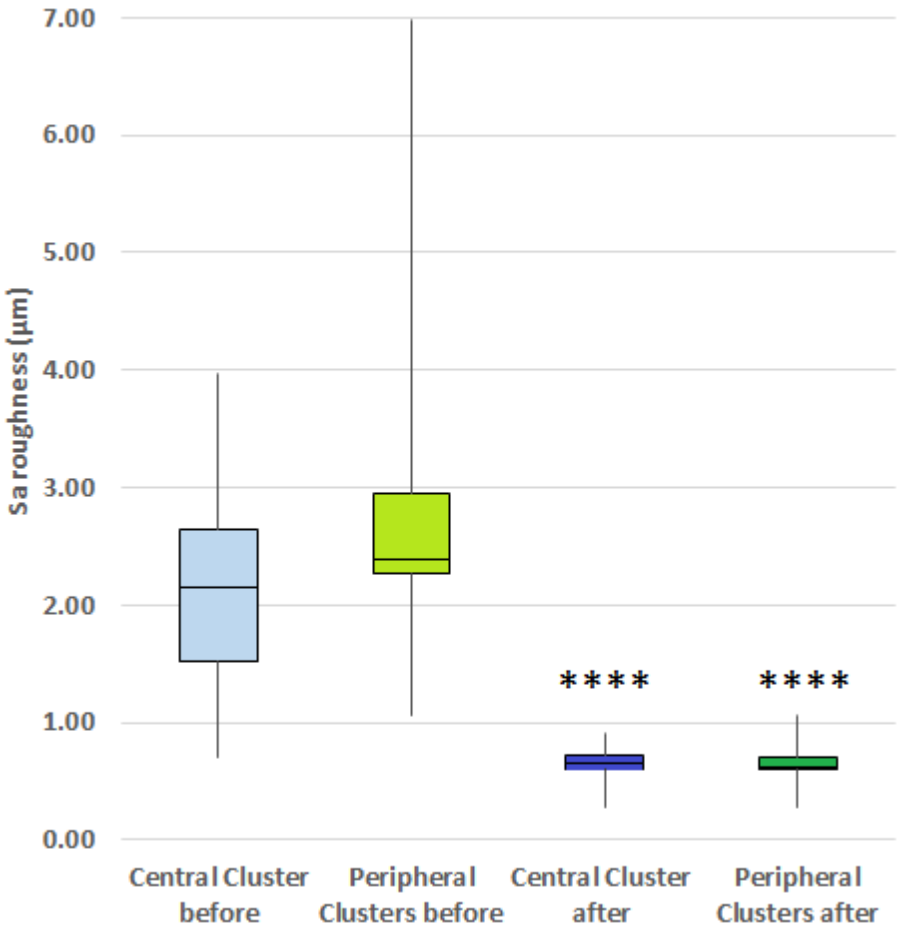


Figure 40: Boxplot demonstrating Sa roughness of natural enamel before and after erosion measuring a single central cluster vs 4 peripheral clusters. ****=p<0.0001.

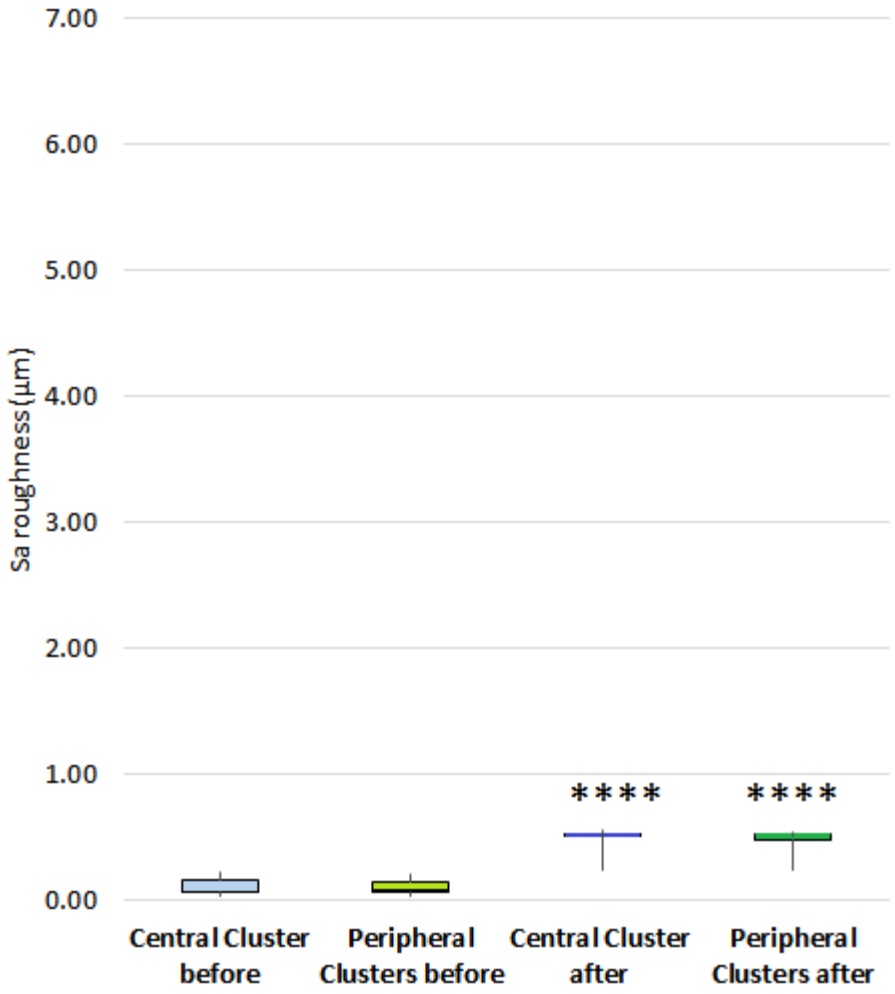


Figure 41: Boxplot demonstrating Sa roughness of polished enamel before and after erosion measuring a single central cluster vs 4 peripheral clusters. ****=p<0.0001

Figure 42 shows representative ESEM images from natural unpolished enamel sample (42A) and a polished enamel sample (42B) before erosion. The natural surface was highly textured at baseline, with perikymata, hexagonal prisms, pits and deeper fissures visible throughout the sample. Whereas the polished enamel had virtually featureless appearance at baseline with the exception of scratch marks left by the polishing regime.

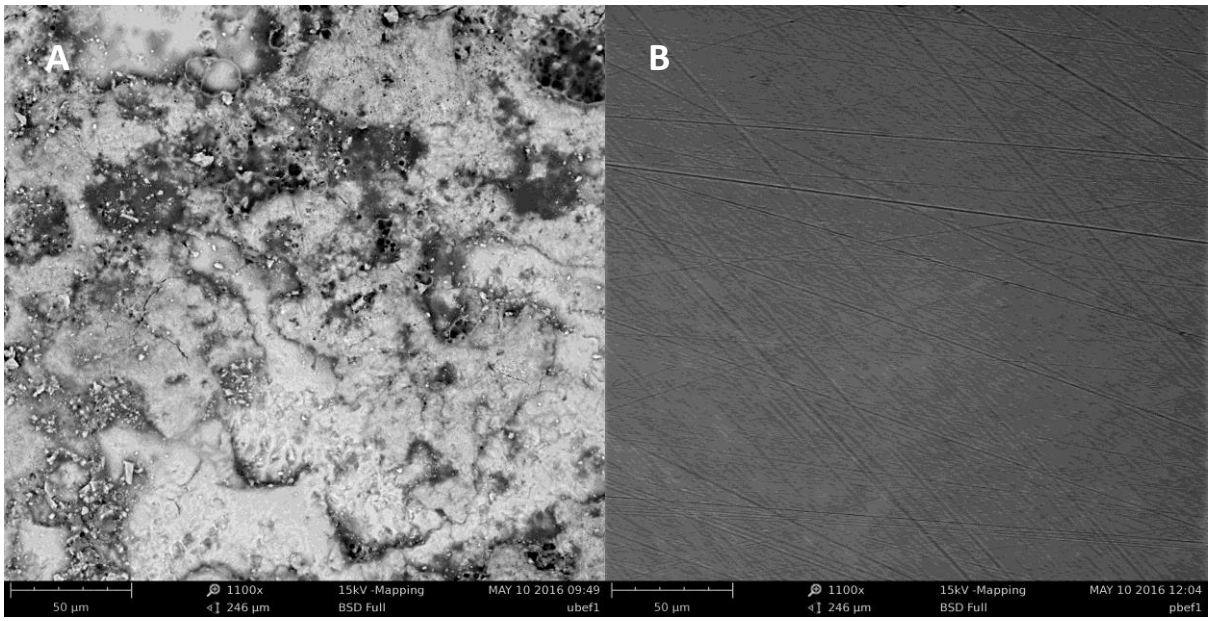


Figure 42: Representative ESEM images before erosion (A) Central natural unpolished enamel sample (B) Central polished enamel (both 1100 X magnification).

Figure 43 shows representative ESEM images of the central and peripheral areas (0.06 mm^2) of natural (43A, B) and polished enamel (43C, D) samples after erosion. These images revealed the presence of similar textural features including enamel prisms and perikymata regardless of whether the images were taken from central or peripheral location for both natural and polished enamel following erosion. The images of the natural unpolished enamel showed a less homogenous appearance, with variations of the number of exposed prisms and varying striations of perikymata within an imaged area but with similar characteristics observed between the central cluster and peripheral clusters (Figure 43A & B).

The images of polished eroded enamel clearly demonstrated the characteristic honeycomb appearance, where the core of the enamel prisms has been dissolved by acid, and the adjacent interprismatic areas appearing more pronounced creating a typical appearance of type 1 enamel dissolution (Figure 43C & D).

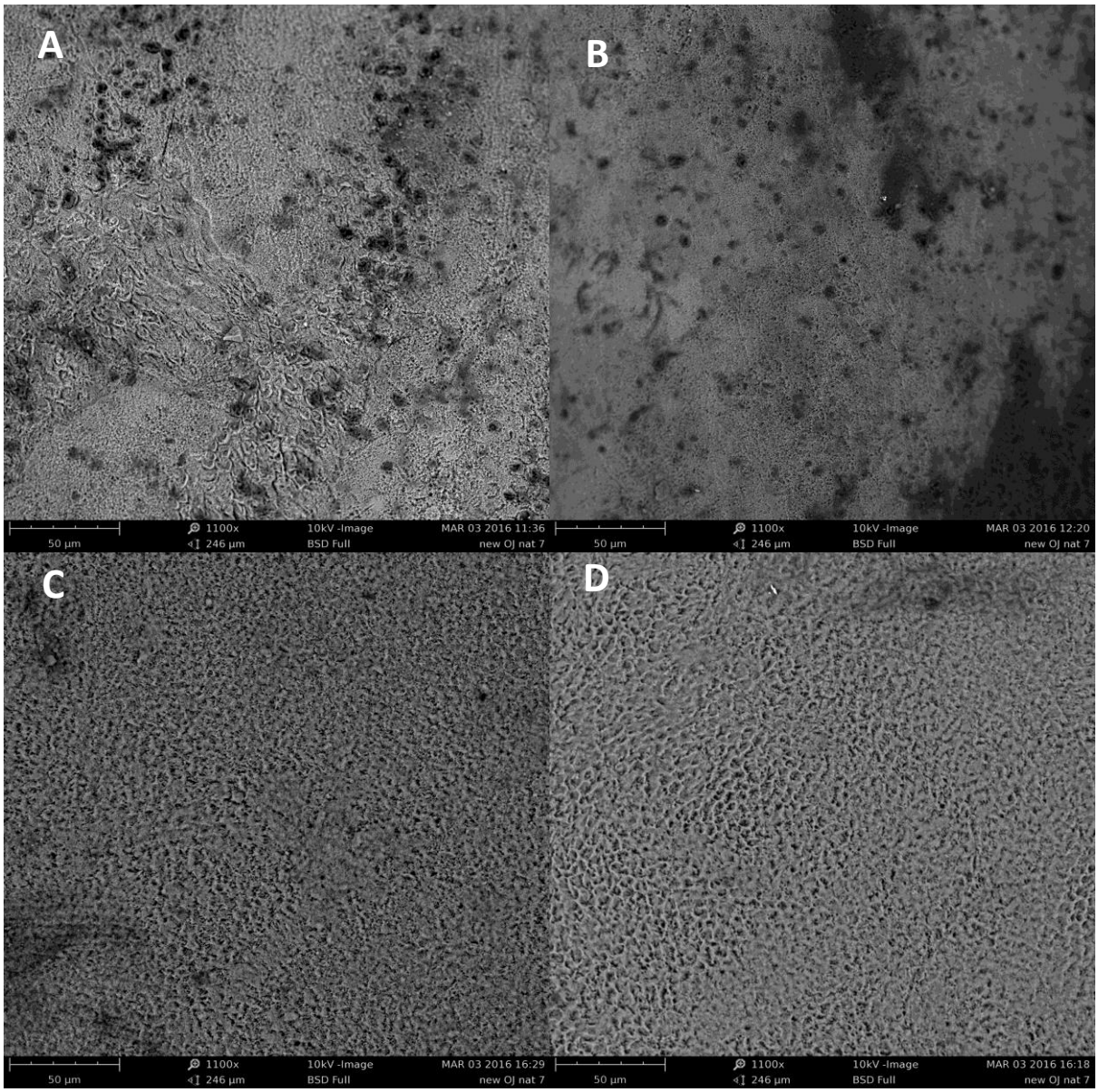


Figure 43: Representative ESEM images after erosion (A) Central natural unpolished enamel sample (B) Peripheral natural unpolished enamel (C) Central polished enamel and (D) peripheral polished enamel (all 1100 X magnification).

4.6 Discussion

Measuring Sa roughness from a single central cluster showed no statistical difference to measuring Sa roughness from peripheral areas for both natural unpolished and polished enamel ($P>0.008$) and so rejects the null hypothesis. Furthermore, both natural unpolished and polished enamel exhibited significant changes in Sa roughness following immersion in dietary acid with natural unpolished enamel becoming significantly smoother ($P<0.0001$) and polished enamel becoming significantly rougher ($P<0.0001$). Therefore, the central cluster could be considered representative of the whole surface and would make future measurements more convenient.

In Chapter 2 it was identified that baseline values of unpolished natural enamel tended to be variable, it was uncertain if this would affect Sa roughness over different locations of the natural unpolished enamel samples. Furthermore, it was previously suggested that erosive changes varied over different locations on the surface of natural unpolished enamel. Meurman and Frank (1991) examined qualitative effects of erosive wear on polished and natural unpolished enamel and identified variations in erosion pattern which they linked to structural variations within natural unpolished enamel, aprismatic enamel specifically. Ganss et al. (2000) compared erosion depths of natural unpolished enamel samples sourced from buccal, distal, mesial and lingual sides. The samples had nail varnish painted upon one half to act as a reference marker and they were immersed in citric acid for 3 hours. Following which the nail varnish and the surfaces were mapped, using a contact profilometer, to measure the lesion depth. They identified differences between lesion depths formed on the different tooth sides and suggested that natural unpolished enamel exhibited a different response to erosion over different locations. However, when measuring Sa roughness, the results suggest that this is not case. Both of these previous studies were performed at a profile level and used a more erosive regime. Surface roughness measurements are recommended for identifying early erosion, which in theory is before structural breakdown at a profile level (Joshi et al. 2016). Therefore, it is possible that whilst profile measurements may differ over different locations, Sa roughness (which is an overview of the overall roughness of a surface) is more consistent over different locations (Leach et al. 2008).

This study compared Sa roughness from a central zone to four peripheral zones which provided an estimate of the overall surface features of the enamel sample, as the curvature of the natural unpolished enamel samples prevented the entire surface being scanned in one setting. Therefore, this suggested that roughness was not location dependent unlike profile. This contradicts previous work which mapped surface topography using (2D) Ra. Zhang et al. (2000) investigated Ra roughness and claimed there were differences in Ra over different locations. However, they compared measuring a 0.5 mm area against 2.5 mm area therefore the differences could be accounted for by the differences in measurement technique and did not justify their conclusion. To be certain of differences in overall roughness of different locations the same measurement technique is required to assess each location as it was done in this current study. The ESEM images for both natural unpolished and polished enamel also showed similar features whether located in the central or peripheral cluster of the samples, which again implicated that imaging the central zone was indicative of the overall sample. The SEM images of natural unpolished enamel from the peripheral zones indicated the difficulties of measuring curved surfaces as the outer corners were slightly out of focus compared with the centre of the images.

Furthermore, this study identified that polished enamel became rougher following erosion which followed the same trend identified in other erosion studies which investigated polished enamel (Austin et al. 2016; Mann et al. 2014). Whereas, in contrast, the natural unpolished enamel became smoother. This result corresponded with previous pilot studies in Chapter 2 and other published work (Arnold et al. 2015; Hara et al. 2016; Mullan et al. 2017b). Surface roughness is quantified by the presence of height deviations from the form of a surface. Surfaces with increased height deviations result in rougher values (Field et al. 2010). Natural unpolished enamel had a relatively rough surface at baseline relating to the natural microscopic characteristics, the reduction in roughness after erosion suggests a reduction in these characteristics. This suggestion was supported by the qualitative ESEM images. When comparing the representative ESEM images of uneroded natural unpolished enamel and the eroded natural unpolished enamel from this study there was visible evidence of a breakdown

in structure. ESEM images show the profile of the surface and not the roughness. It therefore, seems possible that there has been structural breakdown at a profile level and the roughness changes identified are occurring within these areas of tissue loss. Dimensional metrology scientists refer to this as areas of relief, where there is an overall reduction in roughness in areas of tissue loss (Scott et al. 2005).

4.7 Conclusion

The null hypothesis was rejected. Measuring the central zone of natural and polished enamel samples was representative of the overall sample. Natural unpolished and polished enamel both exhibited changes in Sa roughness following erosion in dietary acid, but behaved differently. Natural unpolished enamel became smoother following erosion and exhibited non-significant natural variation in the response to erosion, whilst polished enamel became rougher in a more uniform and less variable manner.

Chapter 5 *In vitro* quantification of surface roughness changes of polished and natural unpolished enamel after different erosion exposures comparing two profilometers of differing resolution

5.1 Introduction

The previous investigations identified significant changes in Sa roughness of natural unpolished and polished enamel after immersion in dietary acid for 45 minutes. However, a minimum erosion time for changes to be detected had not been determined. Furthermore, a previous collaborative study with dimensional metrology scientists to investigate the application of surface roughness measurements to identify changes in the surface of human enamel following acid mediated erosion suggested that high resolution scanning equipment with a lateral resolution of less than 2.5 µm was required for identifying changes in surface roughness of enamel (Austin et al. 2016). However, the red laser confocal profilometer used in Chapters 2, 3 and 4 had a lower lateral resolution than these recommendations. This questions the level of resolution required to identify surface roughness changes on enamel following acid induced erosion, particularly as innovations in digital technology has led to intra oral scanners becoming increasingly used in clinical dentistry.

Intra-oral scanners use common metrological principles present in high-resolution equipment including confocal, triangulation and interferometry (van der Meer et al. 2012). Intra-oral scanners are capable of replacing the need for physical impression taking and casting by using digital technology (Christensen 2008; Christensen 2009; Patzelt et al. 2014). The lateral resolution of intra-oral scanners is roughly quoted as around 10 µm, therefore four times inferior resolution in comparison to the previously recommended optimal resolution for surface texture measurement of enamel of 2.5 µm (Austin et al. 2016).

Before investigating intra-oral scanners, further *in vitro* work using conventional laboratory digital scanning equipment such as a non-contact profilometer, is required to assess what minimum level of

resolution is required to detect changes in the surface roughness of natural and polished enamel following acid induced erosion.

5.2 Aims

- To investigate and determine the minimum time of immersion in acid to detect changes in surface roughness for polished and unpolished enamel.
- To test a method for a future *in situ* study.
- To compare Sa roughness measurements from different resolution confocal profilometers.

5.3 Null Hypotheses

- Surface roughness of natural unpolished enamel and polished enamel will not change following 15, 30 or 45 minutes immersion in dietary acid *in vitro*.
- Sa roughness measurements from different resolution confocal profilometers differ in measurements of eroded enamel.

5.4 Methods

5.4.1 Sample preparation

30 natural unpolished enamel samples and 30 polished enamel samples were prepared in bisacryl composite as previously described in Chapter 2 sections 2.6.1.1 and 2.7.1.1 and randomly allocated into 3 groups (n=10 natural unpolished and n=10 polished per group).

5.4.2 Erosion regimes

Three different three-cycle erosion regimes were investigated. In Group 1, natural unpolished (n=10) and polished (n=10) enamel samples were immersed in 100 mL of the orange juice drink (Sainsbury's Basic pH 3.2 TA 41.3 mmol/ L) for 5 minutes under constant agitation at 62 rpm and rinsed with deionised water by spraying the samples from a water bottle held approximately 5 cm from the samples for 30 seconds, and the process repeated two times resulting in a total erosion time of 15 minutes (Mistry et al. 2015). In Group 2, an equal number of samples were immersed for 10 minutes

per cycle resulting in a total erosion time of 30 minutes and in Group 3 the immersion time was 15 minutes per cycle resulting in a total erosion time of 45 minutes as shown in Figure 44.

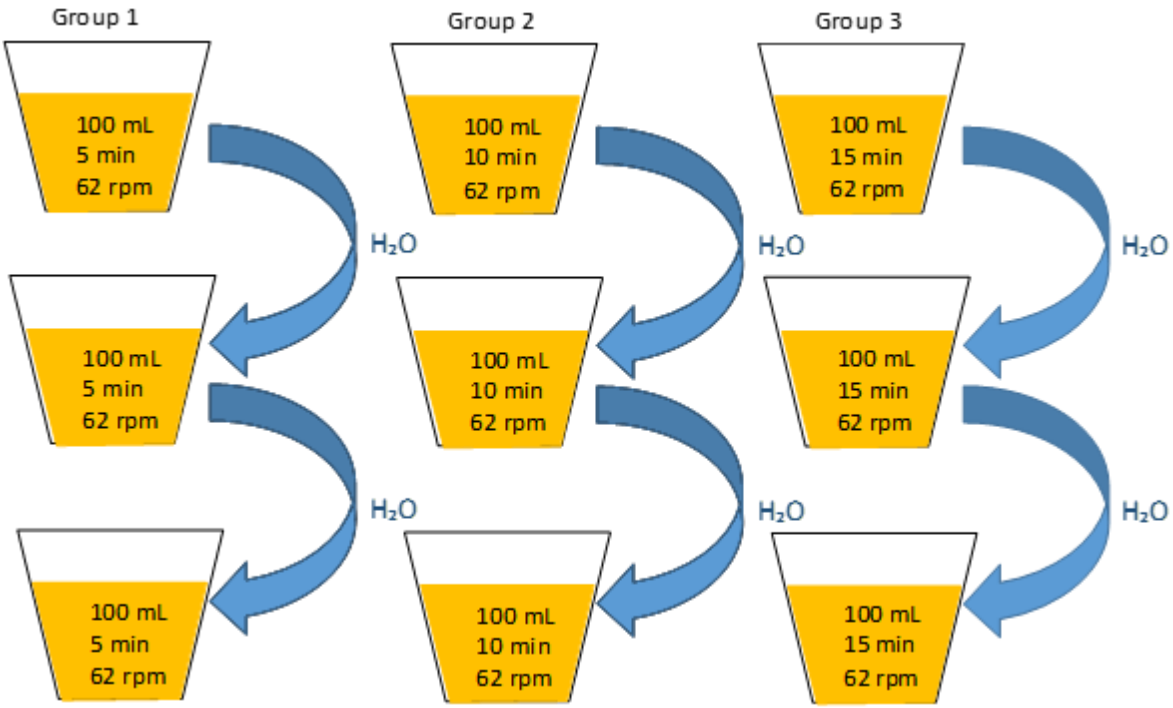


Figure 44: Three erosion regimes were investigated 3x 5 minutes, 3x 10 minutes and 3 x 15 minutes immersion in 100 mL of orange juice under agitation at 62 rpm.

5.4.3 Surface roughness image acquisition and analysis

Part A,

All groups (natural unpolished and polished) were imaged at baseline and after three cycles of the erosion with the white light profilometer (Xyris 4000, TaiCaan, Southampton, UK) using Stages software (TaiCaan, Southampton, UK). Five areas (each 0.04 mm²) were selected from the centre of each sample and scanned at a scanning interval of 4 µm. The resulting images were analysed using BODDIES analysis software (TaiCaan, Southampton, UK). The images were levelled, a 25 µm Gaussian filter applied and Sa roughness extracted.

Part B,

The natural unpolished enamel samples from Group 2 were also imaged at baseline and after completion of erosion cycling using the red laser confocal profilometer and analysed for Sa roughness using MountainsMap (DigitalSurf, France) surface analysis software using methods previously described in Chapter 2 section 2.5.1.3. The white light scan images for the natural unpolished enamel samples from Group 2 were selected and re-analysed with MountainsMap to compare the two measurement devices.

5.4.4 SEM

One representative sample from each group was selected and imaged at baseline and after erosion as previously described in Chapter 4 section 4.4.3.2.

5.4.5 Statistical analysis

The sample size was decided based on previous studies and collaboration with a statistician at King's College. From previous pilot work comparing the correlation of surface roughness with other markers of erosion (enamel microhardness), at 5 % level of significance, to test the null hypothesis of correlation between roughness and microhardness as 0.5 against an alternative of 0.765, required a total sample of 20 to achieve the power of 80% to test the significance of correlation, assuming the bi-variate normal model. The power calculation was carried out using Gpower version 3.1.5.

SPSS 23 was used to analyse the data. Kolmogorov-Smirnov, Shapiro-Wilk tests and histogram plots were used to assess normality. The data comparing the three erosion times were not normally distributed therefore Independent Kruskal Wallis one way analysis on ranks were used to compare data amongst the groups at baseline and after erosion with paired Mann-Whitney Rank Sum and post hoc Dunn's tests to compare groups individually before erosion versus after erosion. However, the

data comparing the two devices for Group 2 were normally distributed therefore means and standard deviations were used to express the data and paired T Tests performed. Significance was set at $p<0.05$.

5.5 Results

Part A

The median (IQR) Sa roughness of natural unpolished enamel samples in Group 1 (3x 5 min) was 0.65 (0.30) µm at baseline which decreased to 0.49 (0.35) µm after erosion, however the difference was not statistically significant. The median (IQR) Sa roughness of natural unpolished enamel samples in Group 2 (3x 10 min) was 0.48 (0.38) µm at baseline which decreased to 0.44 (0.2) µm after erosion and again the difference was not statistically significant. The median (IQR) Sa roughness of natural unpolished enamel samples in Group 3 (3x 15 min) was 0.50 (0.29) µm at baseline which significantly decreased to 0.42 (0.14) µm after erosion ($p<0.05$). There were no statistically significant differences between the three groups at baseline ($p>0.05$). The full results are shown in Table 14 and Figure 45

The median (IQR) Sa roughness of polished enamel samples in Group 1 (3x 5 min) was 0.08 (0.10) µm at baseline and 0.26 (0.02) µm after erosion ($p<0.001$), Group 2 (3x 10 min) was 0.15 (0.11) µm at baseline and 0.25 (0.07) µm after erosion ($P<0.001$) and Group 3 (3x 15 min) was 0.10 (0.08) µm at baseline and 0.27 (0.04) µm after erosion ($P<0.001$). There were no statistically significant differences between the groups at baseline ($p>0.05$) and between the roughness changes of the three groups ($p>0.05$).The full results are shown in Table 14 and Figure 46.

Table 14: Median (IQR) Sa roughness of natural unpolished and polished enamel at baseline and after either 3x 5minutes, 3x 10 minutes or 3x 15 minutes of erosion in orange juice measuring with white light confocal profilometery. Roughness change is expressed where there have been significant changes. ^{ns} = P>0.05,*= p<0.05, **=P<0.01, ***=p<0.001

Natural unpolished enamel			
Erosion time	**Baseline Sa (µm)**	**Post erosion Sa (µm)**	**Roughness Change (µm)**
3x 5min	0.65 (0.30)	0.49 (0.35) ^{ns}	0
3x 10min	0.49 (0.38)	0.44 (0.2) ^{ns}	0
3x 15 min	0.50 (0.29)	0.42 (0.14) *	-0.14 (0.34)
Polished enamel			
Erosion time	**Baseline Sa (µm)**	**Post erosion Sa (µm)**	**Roughness Change (µm)**
3x 5 min	0.08 (0.10)	0.26 (0.02) ***	0.17 (0.13)
3x 10 min	0.15 (0.11)	0.25 (0.07) ***	0.12 (0.09)
3x 15 min	0.10 (0.08)	0.27 (0.04) ***	0.18 (0.15)

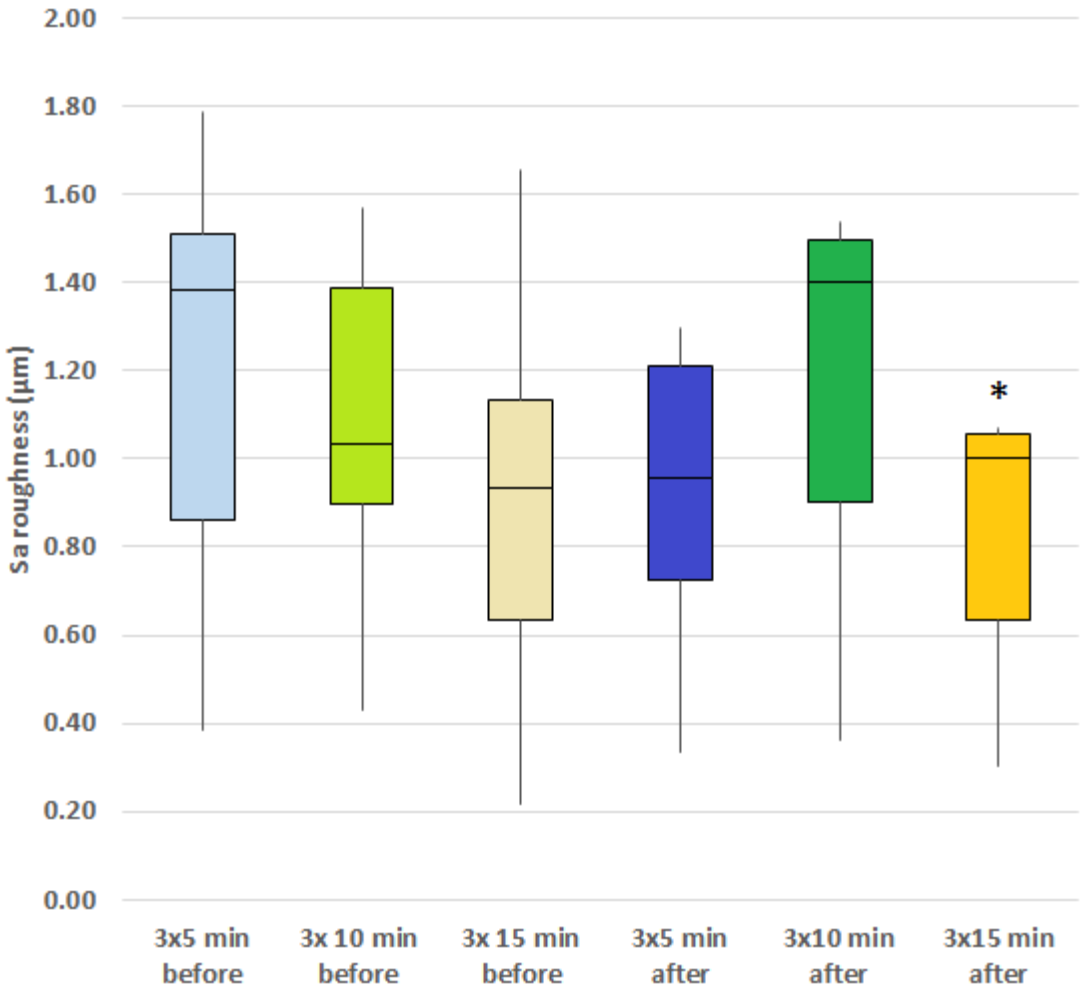


Figure 45: Boxplot demonstrating Sa roughness of natural unpolished enamel before vs. after erosion (either 3x 5 minutes, 3x 10 minutes or 3x 15 minutes) *=P<0.05

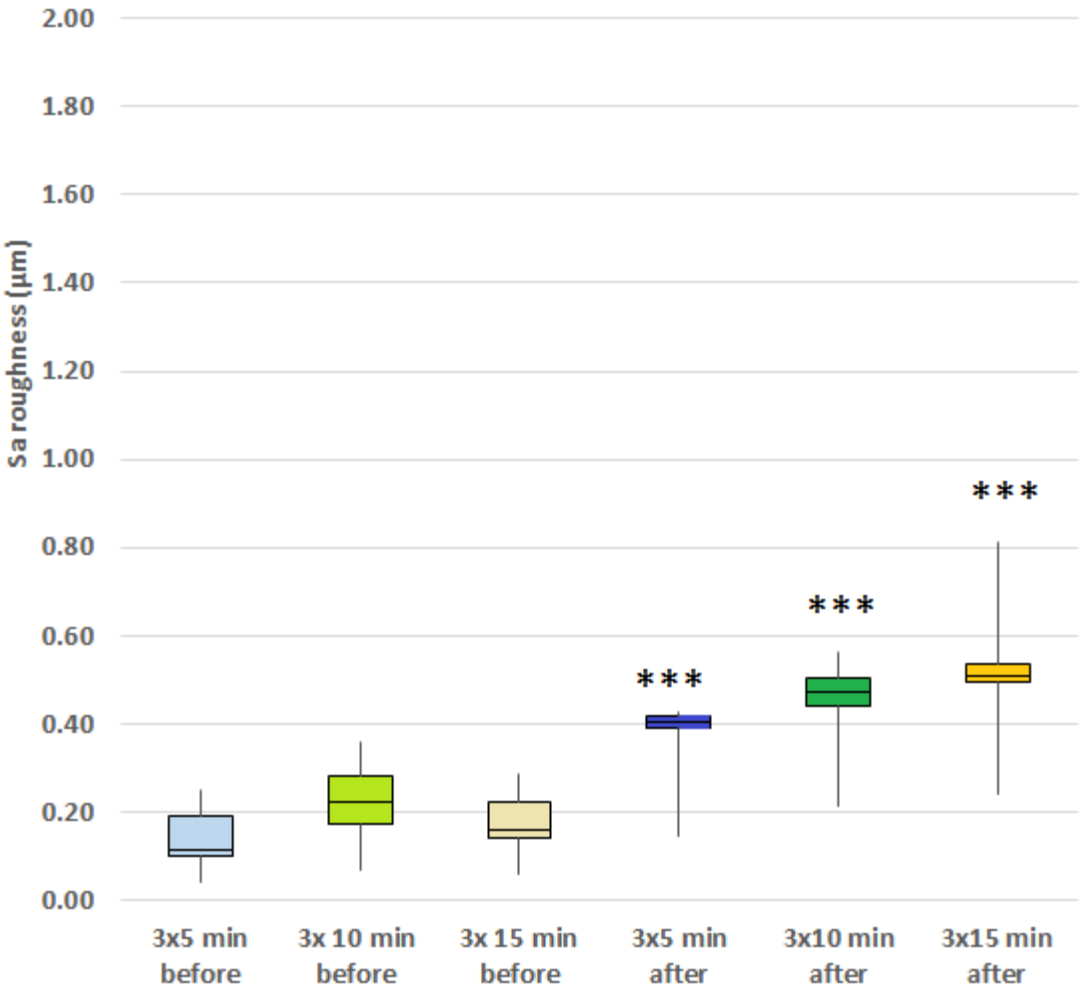


Figure 46: Boxplot demonstrating Sa roughness of polished enamel before vs after erosion (either 3x 5 minutes, 3x 5 minutes or 3x 15 minutes) ***=P<0.001

The representative ESEM images for before and after erosion for natural unpolished enamel are shown in Figure 47. There was little visual difference from the image at baseline (47A) and the image following 15 minutes immersion in orange juice (47B). However, after 30 minutes of immersion there was an increase in the number of exposed prisms and a generalised flattening of the surface (47C). After 45 minutes immersion (47D) there was structural breakdown of the surface.

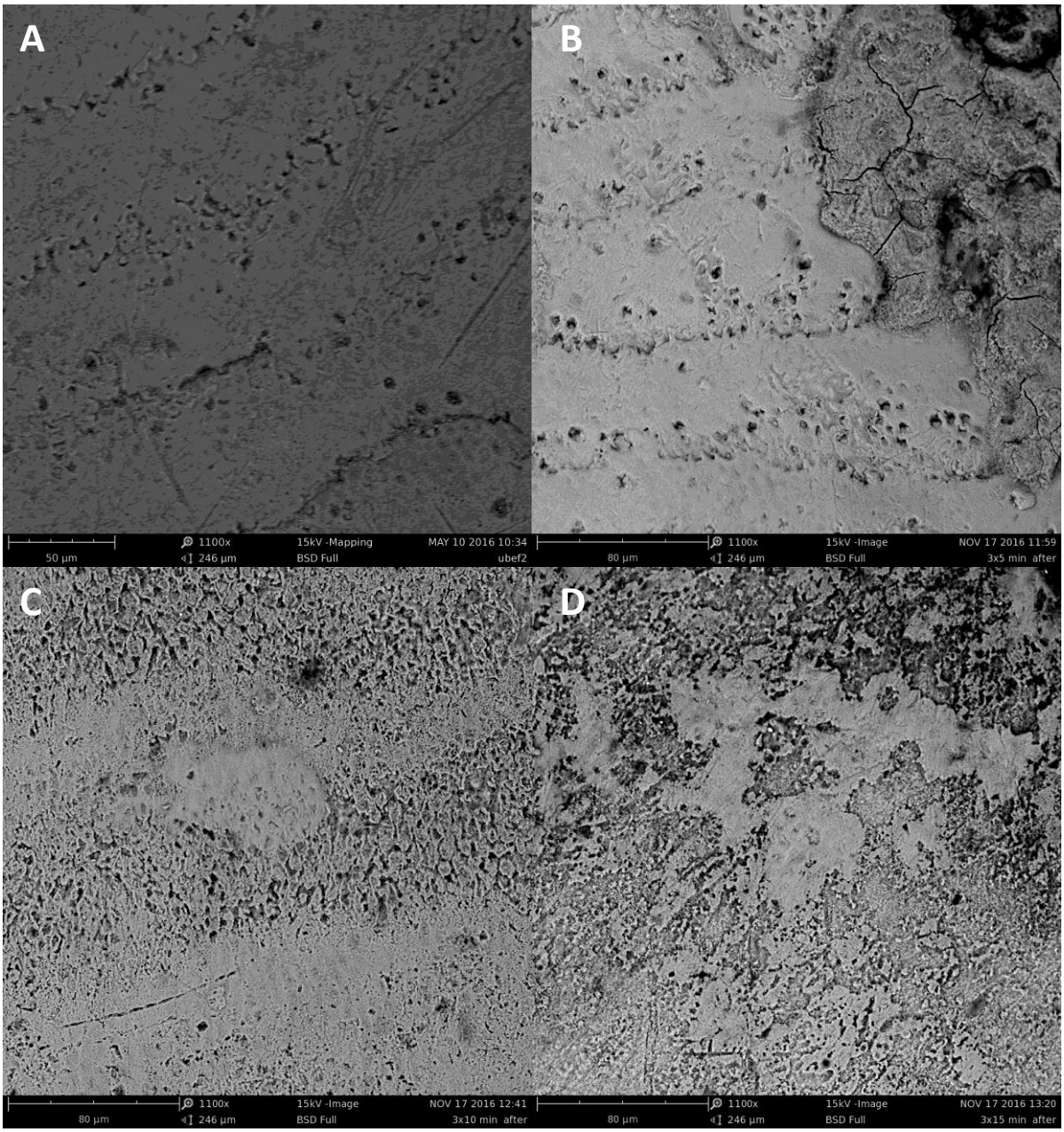


Figure 47: ESEM images of natural unpolished enamel at baseline (A), from Group 1 after 15 minute of erosion (B), from Group 2 after 30 minutes of erosion (C) and from Group 3 after 45 minute of erosion (D) (all 1100 X magnification).

The ESEM images before and after erosion of polished enamel are shown in Figure 48. The baseline image (48A) is virtually featureless showing the scratch marks from the polishing regime. The typical honeycomb appearance, where the core of the enamel prisms has been dissolved by acid and the adjacent interprismatic areas, was evident after 15, 30 and 45 minutes of erosion with little difference between the erosion times (48B, C and D).

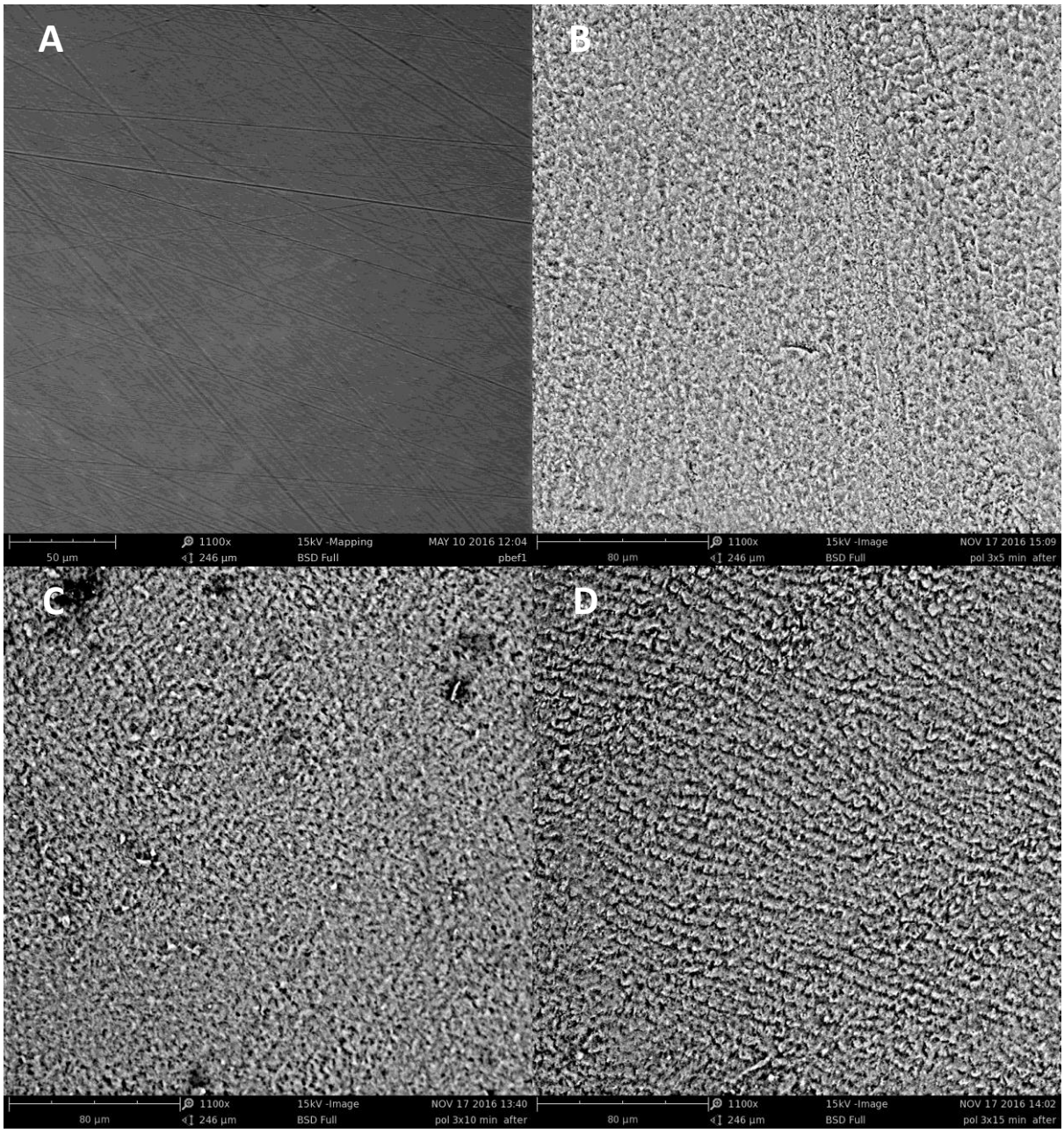


Figure 48: ESEM images of polished enamel at baseline (A), from Group 1 after 15 minutes of erosion (B), from Group 2 after 30 minutes of erosion (C) and from Group 3 after 45 minute of erosion (D) (all 1100 X magnification).

Part B

The results of the Sa roughness of Group 2 (at baseline and following a total of 30 minutes erosion) with the red laser profilometer and the white light profilometer are shown in Table 15 and Figure 49. The mean (SD) of natural unpolished enamel measured with the red laser was 0.35 (0.06) µm which decreased to 0.31 (0.05) µm after SEM images of polished enamel at baseline (A), from Group 1 after 15 minutes of erosion (B), from Group 2 after 30 minutes of erosion (C) and from Group 3 after 45 minutes of erosion (D) (all 1100 X magnification). However, this difference was not significant. The mean (SD) of the white light was 0.61 (0.23) µm which decreased to 0.60 (0.11) µm and this difference was not significant ($p>0.05$). There was no statistical difference between measurements the red or the white lasers ($p>0.05$).

Table 15: Mean (SD) Sa roughness before and after 30 minutes of erosion red laser vs white light profilometer. NS= P>0.05

Profilometer	Baseline Sa (µm)	Post erosion Sa (µm)
Red laser	0.35 (0.06)	0.31 (0.05) ^{NS}
White light	0.61 (0.23)	0.60 (0.11) ^{NS}

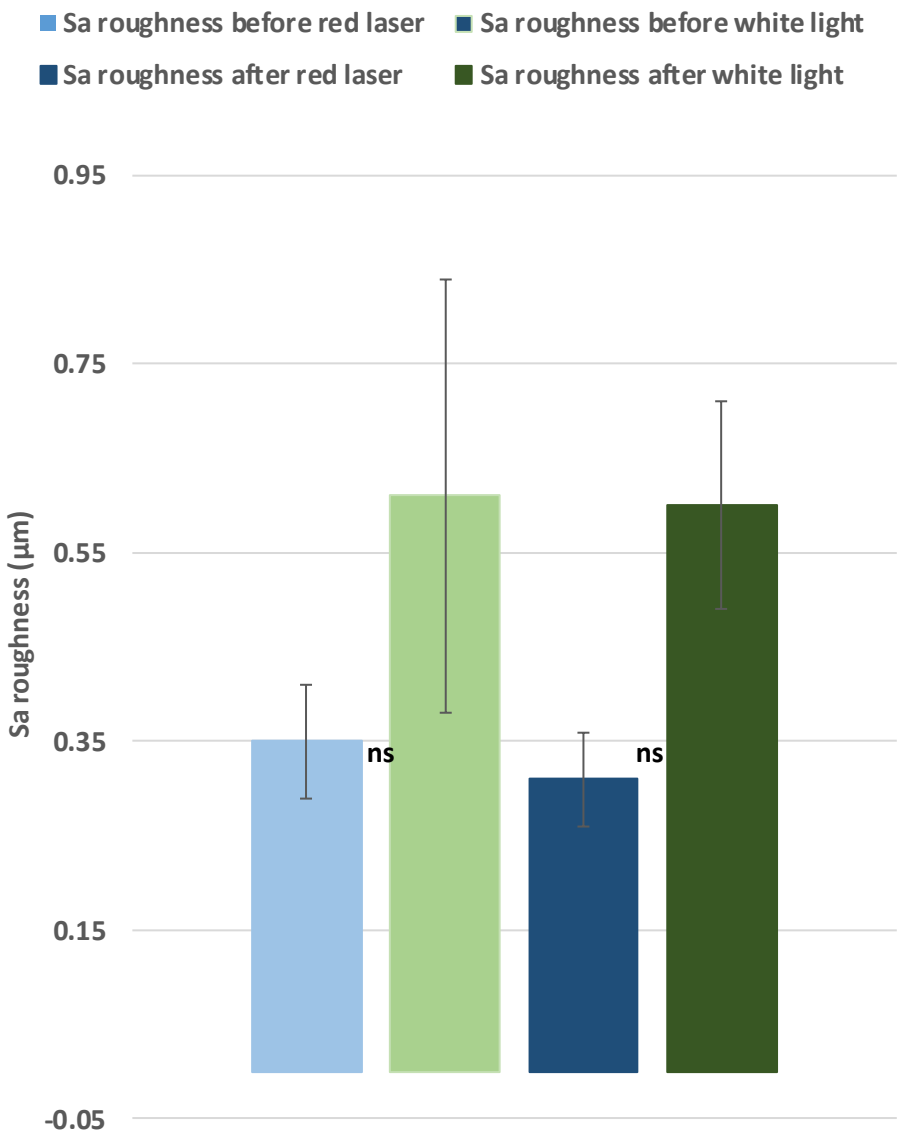


Figure 49: Mean (SD) Sa roughness before and after 30 minutes of erosion red laser vs white light profilometer. NS= P>0.05

5.6 Discussion

This study identified changes in Sa roughness of polished enamel after 15 minutes in orange juice whereas for natural unpolished enamel samples it took 45 minutes for changes to be detected. There was also more variability in the data for natural unpolished enamel, so whilst a statistical change was identified this needs to be taken into consideration. The null hypothesis was rejected for polished enamel and partially rejected for natural unpolished enamel. This suggests that natural unpolished

enamel is more resistant to erosion and its outer surface, containing minerals or different structural integrity, may provide the resistance to the effects of citric acid.

Scanning with the white light profilometer identified statistically significant increases in Sa roughness of polished enamel after 15, 30 and 45 minutes but there were no statistically significant differences in overall roughness changes and suggests there was saturation of the roughness change after 15 minutes. Previously Chapter 2 identified saturation of surface roughness changes of natural unpolished enamel after 40 minutes of immersion in orange juice.

Although previous *in vitro* erosion studies have identified a linear relationship between increased step height loss and increased exposure time (Zheng et al. 2009; Jager et al. 2012) this does not translate to changes in surface roughness as shown in this study and Chapter 2 section 2.3.2. Mann et al. (2014) also identified a similar trend in dose response with changes in Sa roughness of polished enamel. Polished enamel samples were eroded in HCL for 30, 60 and 120 seconds with Sa roughness measured at baseline and after each immersion time. They identified significant increases in Sa roughness from baseline after each immersion time. However, there were no statistical differences between the three times, which suggested that surface roughness changes saturate more readily and caution must be undertaken between linking roughness measurements with other indicators of erosion.

The ESEM images of the polished enamel compared well with the saturation of roughness values. The uneroded polished enamel image was virtually featureless but after erosion the core of the enamel prisms had been dissolved by acid and the adjacent interprismatic areas appeared more pronounced creating the characteristic honeycomb appearance, this occurred after 15, 30 and 45 minutes of erosion with little differences amongst the images at these different erosion times. Roughness measurements are recommended, like microhardness, for early erosion and this observation of saturation would support this recommendation.

However, what defines an appropriate immersion time to determine early erosion which will result in quantifiable changes for both polished and natural unpolished enamel is difficult to determine. In the case of polished enamel 15 minutes immersion in orange juice was adequate, however, 45 minutes was required for natural unpolished enamel. Therefore, the three erosion times were maintained for the clinical *in situ* study in Chapter 6 to optimise roughness measurement for both surfaces.

Natural unpolished enamel requiring a minimum immersion time of 45 minutes indicated that natural unpolished enamel was less susceptible to erosion compared with polished enamel. This is supported by previous studies. Meurman and Frank (1991) investigated erosive effects on polished and natural unpolished enamel samples with SEM imagery and showed that polished enamel samples had an increased response to erosion compared with polished enamel. Ganss et al. (2000) investigated lesion depth using contact profilometry on polished and natural unpolished enamel samples following erosion in citric acid for 3 hours. They identified significant increases in lesion depths for polished enamel compared with natural unpolished enamel. Hara et al. (2016) et al. also suggested natural unpolished enamel was less susceptible to erosion when they identified changes in Sa roughness of polished enamel but not natural unpolished enamel. It has been suggested that it is the removal of the outer layer of enamel which predisposes polished enamel to the effects of erosion. This outer layer contains aprismatic enamel which is credited as being the main source of resistance. Assessment of SEM images has identified that the regions of aprismatic enamel are less affected by the acid compared with prismatic enamel where the core of the prisms are hollowed out leaving the interprismatic regions protruding to create the typical honeycombed appearance (Barbour & Rees 2004). The surface roughness at baseline for polished enamel was consequently very smooth and despite becoming rougher after erosion, eroded polished enamel was still smoother than natural unpolished enamel. This relates to the natural unpolished enamel having more variable characteristics in its structure. Studies often refer to enamel with a large variation of crystalline deviation as being highly textured (Al-Jawad et al. 2007). However, as well as being less susceptible to erosion the *in vitro*

studies in this thesis indicated that natural unpolished enamel behaved differently compared with polished enamel, becoming smoother after up to 60 minutes of erosion in orange juice. Hara et al. (2016) identified natural unpolished enamel became significantly smoother after immersion in citric acid for a total of 8 minutes.

In this study, the overall form of the natural unpolished enamel surface remained intact even after 15 and 30 minutes of erosion. However, after 45 minutes of erosion there was evidence of structural breakdown and increase in the proportion of erosive prismatic features eliminating longer wavelength features which dominate the natural unpolished enamel surface such as perikymata and other macro-histological features (Nanci & Ten Cate 2008). Surface roughness is calculated from height deviations, with rougher surfaces having larger deviations (Field et al. 2010). The reduction in the identifiable features on the ESEM images may partially explain the decrease in Sa roughness as areas of relief can occur, which was discussed in Chapter 4. However, caution must be taken when combining investigations at a profile level compared with investigations at a roughness level. Previously, a series of studies suggested a link between reflectometry and surface roughness changes upon the premise that natural unpolished enamel would become rougher following erosion similarly to polished enamel. However, more recent work including studies presented in this thesis would suggest that where early erosion is considered this correlation is not present (Mullan et al. 2016; Hara et al. 2016; Arnold et al. 2015). It must be acknowledged that erosive tooth wear is a cumulative process and a previous study which demineralised natural unpolished enamel samples for 20 hour increments identified roughness increases (Zhang et al. 2000). However, for this thesis the overall aim was to develop a method to quantify surface roughness of natural unpolished enamel following erosion from dietary acid, with the possibility of the method being adapted to be used as a clinical indicator for erosion. Therefore, the intentions were to quantify changes before irreversible bulk tissue loss had occurred and did not investigate further beyond 60 minutes of erosion for this reason.

The difference in susceptibility of the polished enamel samples (where the outer layer has been removed) to natural unpolished enamel (with the outer layer intact) has clinical significance. Potentially any clinical intervention which alters the outer structure of enamel could reduce the innate resistance of that tooth to erosion. Furthermore, once structural breakdown has occurred it could be anticipated that any further erosive wear would proceed at an accelerated rate.

This chapter identified that natural unpolished enamel is more resistant to erosion from both qualitative ESEM images and quantitative results. However, measurement error must be considered for the roughness values. Chapter 3 identified that there were differences in measurement error when measuring curved surfaces, with up to 20 times more variability measuring natural unpolished enamel compared with polished enamel. This principle also applied to measurement with the white light profilometer. Therefore, whilst ESEM images confirm resistance of natural unpolished enamel it is possible that there were changes at a roughness level after 30 or 15 minutes immersion that were unable to be detected due to measurement capabilities. Measurement error throughout this thesis was reduced as much as possible by selecting small scan areas (each 0.04 mm^2) from centre of the samples (apex of the curvature) and levelling the scan images before analysis as outlined in previous chapters. The dedicated software for the profilometer (BODDIES), was used for analysis as there were difficulties for some scans to be translated for the MountainsMap system due to code incompatibilities. However, there was nothing further that could be done to overcome these inherent measurement issues.

The red laser and white light confocal profilometers were both inferior to the minimum resolution of 2.5 µm suggested by Austin et al. (2016). Therefore, Part B of this chapter challenged what level of resolution was required to successfully quantify surface texture of natural unpolished enamel. Lateral resolution is dependent on the size of the light source and the type and shape of the surface measured (Durakbasa et al. 2011). The red laser confocal profilometer was the higher resolution device with a spot size of 2 µm compared with 7 µm for the white light. To compare the capacity of the two

measurement systems the natural samples in the 30 minutes erosion group (Group 2) were selected to be scanned with the red laser confocal profilometer and the results from both white light and red light scanning analysed with MountainsMap (DigitalSurf, France). Group 2 was chosen as Chapters 2 and 4 had already identified that the red laser confocal profilometer was capable of detecting changes in surface roughness of natural unpolished enamel following 45 minutes immersion, however lower erosion times had not been investigated. The white light also identified changes in surface roughness after 45 minutes but not for 30 minutes. Therefore, the objective was to investigate if the higher resolution red laser confocal profilometer was capable of detecting changes after 30 minutes, where the white light could not. Scanning with the red laser there was also no change in Sa roughness after 30 minutes of erosion and there were no statistical differences between Sa values whether measuring with the red laser or white light. Therefore, despite the differences in resolution of the two systems and despite that they were inferior to the previous recommended resolution of 2.5 µm by Austin et al. (2016) both were adequate to quantify Sa roughness of natural and polished enamel rejecting the null hypothesis.

Previously, Heurich et al. (2010) compared enamel loss measurements of two contact profilometers and a CLM. The contact profilometers had significant lower resolution than the CLM but, at that time, were the gold standard for measuring tissue loss in erosion studies. They found no statistically significant differences between measurements made with the contact profilometers compared with the CLM. Paepegaey et al. (2013) compared enamel loss measurements using a CLM, contact profilometer and non-contact profilometer, identifying strong correlation among the measurements from all three. Comparison of devices measuring surface roughness of human enamel, and changes in surface roughness of natural unpolished enamel following erosion with dietary acid have not previously been performed. There was a previous study which compared roughness measurements with a stylus and laser profilometer measuring ceramic (Whitehead et al. 1999). They identified a correlation between Ra measurements with the two devices, but not with the other roughness

parameters investigated. However, it is difficult to draw comparisons with this study which used a different substrate and 2D parameters, whilst this thesis used 3D Sa throughout measuring enamel samples.

The ability of a lower resolution device to be capable of identifying changes in Sa roughness of natural unpolished enamel provides promise for the development of a clinical measurement of Sa roughness. The potential for this would be in two ways either the development of a replica technique or advancement in the use of intra oral scanners. A replica technique was previously investigated in Chapter 2 using negative replicas. Intra-oral scanners are becoming increasingly used in dentistry with continued advancements. The resolution of intra-oral scanners is similar to the white light profilometer used in this study. Therefore, theoretically there could be potential for intra-oral scanners being used in the future. However, this could not be investigated in this thesis as at present there is no compatibility between the scanned images from intra-oral scanners and software analysis to be able to quantify roughness parameters. Limandri et al. (2016) recently used specialized software and stereo SEM to transform the imaged surfaces in roughness parameters suggesting that future innovations in analysis software may be able to translate the images from intra-oral scanners. However, a further issue is the measurement error associated with hand held chair side scanning. Chapter 3 detailed the measurement error for the red laser confocal profilometer and the differences between measuring curved versus flat surface. These measurements were recorded under optimal conditions. The profilometer was kept in a temperature controlled room, the samples were placed upon an aluminium platform and the light source emitted at 90˚. The samples remained stable throughout the movement of the stage. In a clinical setting, it would be impossible to emit the light source at 90˚ over each tooth surface and there would be involuntary movement by both operator and patient. Therefore, if the advancements in analysis allow for quantification of surface roughness further work would be required to develop and validate techniques.

For the remainder of this thesis it was decided to continue with the use of the red laser confocal profilometer. Initially when scanning the samples with the white light increased drop out was identified. This was rectified by lowering the sampling rate (intensity Hz) and the samples were rescanned. Theocharopoulos et al. (2010) compared scanning with white light at sampling rates of 1000, 300, 100 and 30 Hz and identified that the least data drop out occurred at 30 Hz. However, the red laser confocal profilometer needed no such adjustments. Furthermore, the data from the white light demonstrated more variability than the data from the red laser. The scans from red laser confocal profilometer were compatible to be used with MountainsMap analysis software which was shown to be highly accurate in Chapter 2. The scan settings developed in this thesis were based upon the red laser confocal profilometer and whilst there were no statistical differences in Sa roughness values detected, theoretically it remains the higher of the resolution of the two with increased capability of detecting small changes.

5.7 Conclusions

The null hypothesis was rejected for polished enamel and partially rejected for natural unpolished enamel. Natural unpolished enamel only exhibited significant changes in Sa roughness following 45 minutes of erosion suggesting lower susceptibility than polished enamel which exhibited quantifiable surface roughness changes after only 15 minutes. There were no differences between surface roughness measurements of natural unpolished enamel with the white light confocal profilometer compared to those from the higher resolution red laser confocal profilometer. Therefore, the null hypothesis was rejected.

Chapter 6 Measurement of surface roughness of natural and polished enamel following exposure to dietary acid *in situ*.

6.1 Introduction

Whilst the previous chapters have identified changes in surface roughness of natural unpolished enamel *in vitro,* further work investigating the effect of natural erosion defences was required prior to any *in vivo* investigations being considered. It has been suggested that the amount of erosion produced in the mouth is approximately one tenth of that produced *in vitro* (West et al. 2011b). This is most likely due to the protective and reparative effects of the pellicle and saliva (Hannig et al. 2005; Loke et al. 2016). Although saliva and pellicle formation can be incorporated into laboratory studies, *in situ* studies are preferred as they provide a closer representation to the clinical scenario and both *ex vivo* and *in vivo* immersion can be investigated simultaneously. Moreover, investigating changes in surface roughness of polished enamel alongside laboratory gold standards such as step height and microhardness provide a benchmark to fully interpret surface changes.

6.2 Aims

- To investigate if changes in surface roughness following exposure to dietary acid for 15, 30 and 45 minutes could be identified in natural unpolished and polished enamel *in situ* comparing *ex vivo* and *in vivo* erosion.
- To investigate microhardness change and step height loss of polished enamel after 15, 30 and 45 minutes of erosion.

6.3 Null hypothesis

- There is no change to the enamel surface following erosion in orange juice.

6.4 Methods

6.4.1 Clinical Study design

This clinical study was a single-blind, randomised intervention study. The operator was blinded to the intervention for all surface roughness measurements, step height measurements and microhardness testing whilst measuring surface changes of natural unpolished enamel and polished enamel following an orange juice acid challenge. To achieve this the samples were coded by a third party and the code was not broken until all analysis had been completed. Ethical approval for the study was granted by the Stanmore Health Research Authority REC ref 15/LO/0417, and the study was conducted using the guidelines for Good Clinical Practice (National Institute for Health Research Clinical Research Network (NIHR), 2016). The study investigated a total 6 erosion regimes divided in 3 different erosion times plus *ex vivo* immersion and *in vivo* rinsing with the same orange juice drink (Sainsbury's Basics, Orange Juice Drink, Sainsbury's Supermarkets Ltd, London pH 3.2 titratable acidity 41.3 mmol OH/L).

6.4.2 Clinical Study population

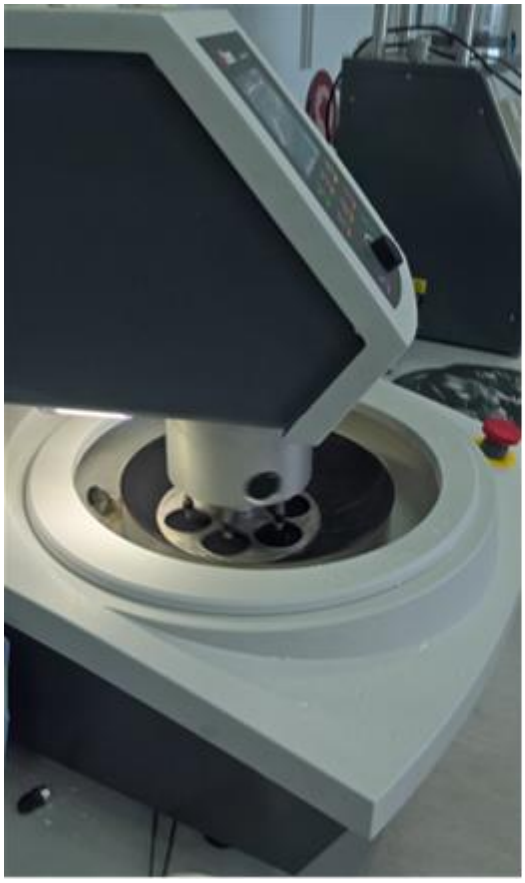
A total of 30 healthy volunteers were recruited from King's College London Dental Institute. Potential participants were approached and informed about the study and given a Patient Information Sheet detailing the study. They were given a minimum of 24 hours to consider the study and if they were interested in taking part they attended a screening appointment in the Oral Clinical Research Unit at King's College London Dental Institute to ensure their eligibility to take part in the study. Those recruited into the study signed consent forms. The inclusion criteria stipulated none to mild erosive tooth wear maximum score of 2 in each sextant and cumulative score no more than 8, aged 18 years and over, willing to participate, not enrolled in any other research, more than 20 anterior and posterior teeth, no active carious lesions and a maximum BPE score of 2 (no periodontal disease). The exclusion criteria stipulated pregnancy or breast feeding, medical history likely to impact on attendance or mobility, insulin dependent diabetes, saliva diagnoses (xerostomia), lower orthodontic appliances, dentine hypersensitivity, defective restoration of the occlusal or incisal surfaces of upper

anterior teeth and first molars and any condition that precluded consumption of 300 mL of orange juice a day for 5 consecutive days.

6.4.3 Sample preparation

Extracted human molars were collected and sectioned to produce buccal enamel sections as described in Chapter 2 section 2.3.1.1 A total of 120 samples, 60 polished and 60 natural unpolished enamel samples, were prepared for the *in situ* study. Unlike the previous studies the enamel samples had to be able to fit into custom made splints to be worn by the participants. Therefore, specific custom mould trays were made as the dimensions of the *in situ* samples needed to be smaller to fit in the custom made splints. To make the moulds 6 aluminium SEM stubs (each with a 12.5 mm diameter) were inserted into silicone duplicating material (Metrosil silicone duplicating material part A and B, Metrodent Ltd UK) held in a circular glass container. For the natural unpolished enamel samples, buccal enamel sections were embedded in bisacryl composite (Protemp4, 3M, ESPE, UK) leaving the outer surface uncovered. They were cleaned using a soft toothbrush and non-fluoridated toothpaste (Kingfisher, Norwich, UK) and the smear layer removed with ethanol. For the polished samples 60 buccal enamel sections were fully embedded in bisacryl composite (Protemp4, 3M, ESPE, UK) and polished flat. A different polishing machine and regime was used, as the previous machine used in the earlier studies had been replaced in the Laboratory. The samples were inserted into the polishing machine's automated polishing head (LaboForce 100, Struers, ApS, Ballerup, Denmark) using platform ring spacers and secured using a customised jig. The 60 samples were then polished using a series of Silica Carbide Grits (Versocit, Struers A/S, Copenhagen, Denmark); Grit size 80 for 3 seconds repeated until the enamel was exposed, size 180 for 6 seconds, 600 for 15 seconds, 1200 for 20 seconds, 2500 for 30 seconds and 4000 for 45 seconds under copious water irrigation. The polishing machine emitted a 10 N force on the centre of the samples and the rotation was conducted by the arm at 50 rpm and the plate at 150 rpm for the full succession of grits. Thus, optically flat enamel samples were pre pared

with an approximate flatness tolerance of 0.4 µm. The polishing machine and grit sequence are shown in Figure 50.



Grit Size	Time (s)
500	5 (repeated until enamel exposed)
1200	10
2000	40
4000	120

Figure 50: Image of polishing machine and grit sequence used for clinical study to produce polished enamel samples.

Following preparation all the samples for the study were ultrasonicated in deionised water for 15 minutes and immersed in sodium hypochlorite prior to baseline measurements being recorded. For the polished samples, PVC tape was applied over the enamel to create a window of exposed enamel as described in Chapter 2 section 2.4.1.1. All samples were immersed in sodium hypochlorite again prior to insertion into the customised splints to ensure disinfection of the samples.

6.4.4 Clinical Study Stage 1: Appliances and wash out

Lower impressions were recorded in alginate using standard stock trays. Custom made lower orthodontic appliances were made by the laboratory to accommodate a total of four enamel (2 left and right hand side) samples positioned buccally in the premolar/molar region as shown in Figure 51 below. The splints were made from a thermosetting silicone and were soft and flexible. Participants were given a non-fluoridated toothpaste (Kingfisher, Norwich, UK) and standard manual toothbrush to use as a washout period for 5 days before the intervention part of the study and were asked to refrain from eating or drinking for two hours prior to the start of the study appointment.

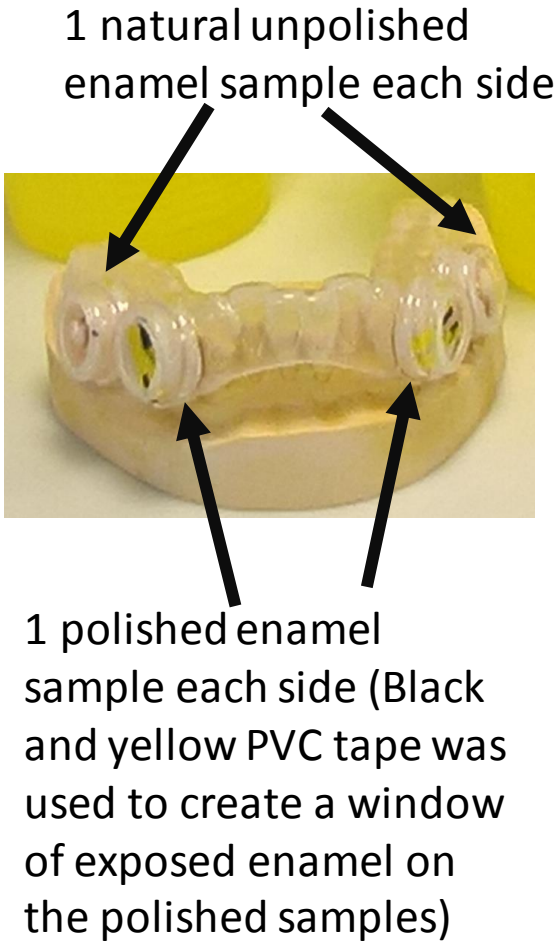


Figure 51: Image of custom made lower appliance containing buccally positioned natural unpolished and polished enamel samples.

6.4.5 Clinical Study Stage 2: Study visit

Participants were randomly allocated into one of 3 erosion times using statistical software. (GraphPad Prism). The erosion times were allocated into; Group 1 for 5 minutes per cycle, Group 2 for 10 minutes per cycle and Group 3 for 15 minutes per cycle. Participants were assigned a code 1 to 30 based upon the sequence of their scheduled visit. Within the software the 'random numbers' function was selected as the analysis tool followed by the option to randomly assign subjects to groups, the number of participants per group (10) was inserted into the software and the number of treatment groups (3). The software assigned codes 1, 2, 3 randomly to the participant number. Each day of the study a coin was flipped to determine which side the samples were to be removed for *ex vivo* immersion with tails for left and heads for right. The participants were unaware of their allocation until they presented for the study visit. At the beginning of the study visit the splints containing four samples (RHS 1 natural unpolished 1 polished and LHS 1 natural unpolished and 1 polished) were inserted and worn for 30 minutes. Following which the enamel samples (1 natural unpolished and 1 polished) were removed from one side to begin the erosion regime as described below. A flow chart of the study visit is shown in Figure 52. The rinsing was conducted under supervision, however during the 30 minute and 60 minute intervals the participants were free to leave the clinical area. After the 3rd and final erosion cycle the participants were given a desensitising toothpaste (Sensodyne Repair & Protect, GSK, Weybridge, UK).

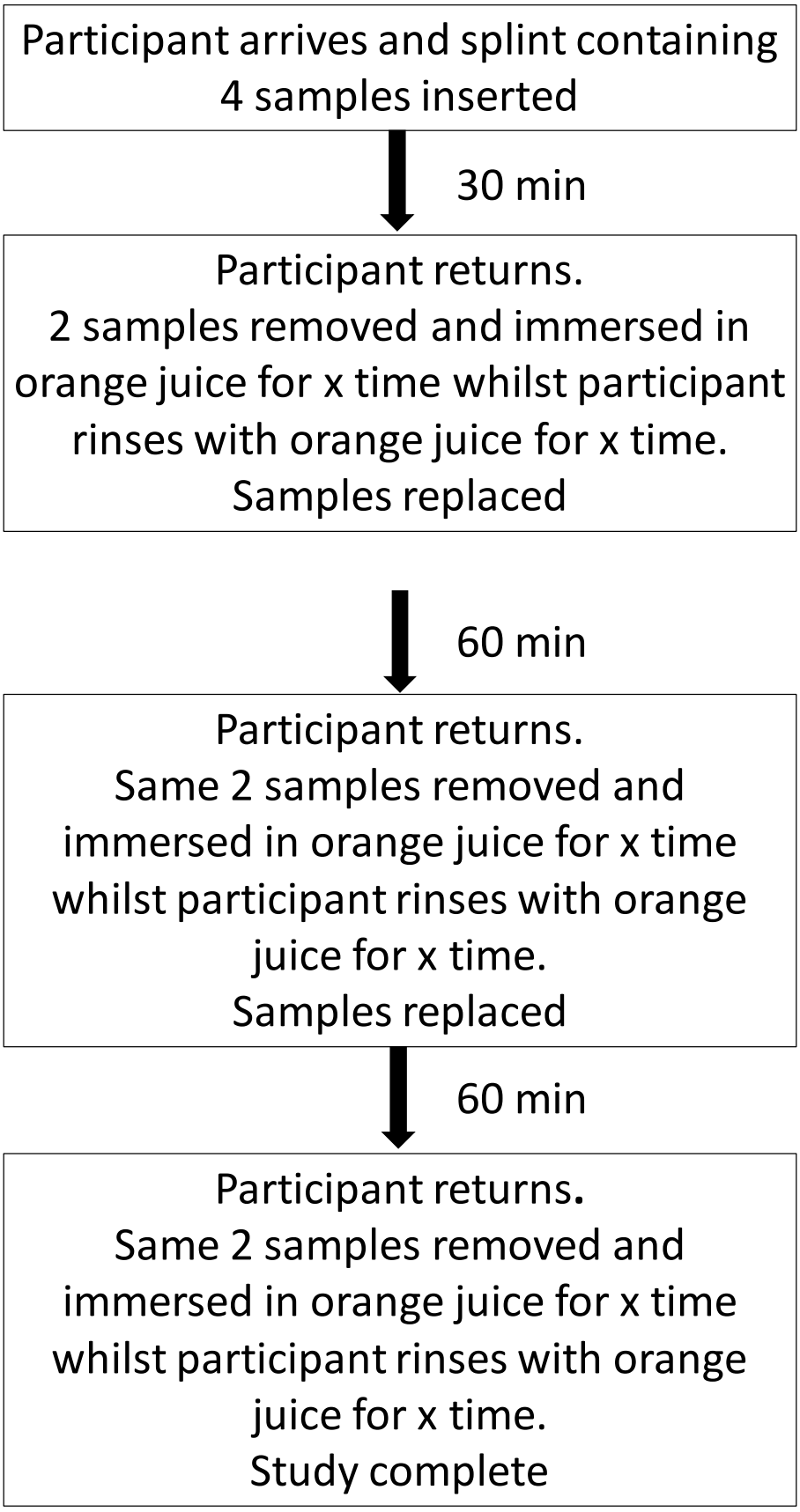


Figure 52: Flow chart of study visit.

6.4.6 Erosion regimes

As stated above, the clinical study investigated six erosion regimes divided in three erosion times plus *in vivo* rinsing in orange juice drink and *ex vivo* immersion in the same orange juice drink (Sainsbury's Basics, Orange Juice Drink, Sainsbury's Supermarkets Ltd, London) pH 3.2 titratable acidity 41.3 mmol OH/L. The erosion times investigated follow the results from Chapter 5. For *in vivo* erosion, each participant was asked to rinse with orange juice (whilst wearing the splint containing two samples, one natural unpolished and one polished) for their allotted time either 5, 10 or 15 minutes. This was standardised by setting up cups with 10 mL of the orange juice and a timer set at 1 minute. The participant was asked to insert the liquid into their mouth at the same time as starting the timer and when the alarm sounded to expectorate and repeat until they completed his/her time allocation of either 5, 10 or 15 minutes. Simultaneously the two *ex vivo* samples (one natural unpolished, one polished) were immersed in 20 mL of orange juice and agitated at 62 rpm for the allocated time using an orbital shaker (Stuart Scientific, Mini Orbital Shaker S05, Bibby). This completed one cycle after which the removed samples were reinserted into the splint which was worn for a further hour after which the rinsing/immersion regime was repeated. The process was repeated a further instance to complete three cycles of erosion.

6.4.7 Image acquisition and analysis

Five areas (each 0.04 mm^2) were conveniently selected from the centre area of natural unpolished and polished enamel samples to be scanned at baseline and after erosion with the red laser confocal profilometer and analysed for Sa roughness as previously described.

A second larger scan was carried out for each polished enamel sample to measure step height after erosion. The light source was centred in the middle of the eroded zone for each sample and the scan settings were set to ensure both reference areas and the eroded zone were scanned equally

(approximately 3 mm by 3mm). The scanning interval was set at 10 µm. The samples were scanned in a raster pattern resulting in approximately 500 data points. The resulting scan images were analysed using BODDIES analysis software (Southampton, UK) as described in Chapter 2 section 2.4.1.3. The data from the six measurements were averaged per sample.

6.4.8 Microhardness testing

Microhardness testing was carried out at baseline and after erosion for polished enamel samples using the Knoop Microhardness Indenter (Duramin-5, Struers Ltd, Rotherham, UK) as described in Chapter 2 section 2.4.1.3. Microhardness testing was not possible for natural unpolished enamel as described in Chapter 2 section 2.4

6.4.9 Statistical analysis

The sample size calculation was described in Chapter 5 section 5.4.5.

SPSS version 22 was used to analyse the data. Normality was checked using Histogram plots and Shapiro Wilk tests. The Clinical Trial data were normally distributed, with any data not originally normally distributed Log transformed. Levene's tests were used to ensure equal variance. ANOVAs with post hoc Bonferroni, Tukey tests and paired T Tests were carried out. Significance was set at $P<0.05$.

6.5 Results

6.5.1 Clinical study population

A total of 40 potential participants were assessed. Of those, 31 were eligible for the study. One participant withdrew from the study after the initial assessment, due to personal circumstances not related to the study. Therefore, a total of 30 participants completed the study with a mean age of 29.6 years (range 20 to 54 years) and female to male ratio of 2:1. Two participants reported sensitivity the day after they had completed the study. One was in the 3x 5 minutes erosion group and the other was

in the 3x 10 minutes erosion group. Application of fluoride varnish (Duraphat®, Colgate®, Colgate-Palmolive, Germany) was provided for both participants with the addition of the application of self-bond (Scotchbond, 3M, USA) to the cervical margins in the upper left quadrant for the participant in the 3x 10 minutes group. The sensitivity fully resolved in 24 hours following treatment for both participants. Adverse events forms were completed.

6.5.2 Quantitative data

For natural unpolished enamel there were no statistical differences between Sa roughness before erosion versus after erosion for any of the groups, shown in Table 16. Groups 1 and 5 exhibited non-significant decreases in mean (SD) Sa roughness from 0.31 (0.14) µm to 0.23 (0.08) µm and 0.25 (0.14) µm to 0.18 (0.08) µm respectively ($p>0.05$). Whereas Groups 2, 3, 4 and 6 exhibited non-significant increases in mean (SD) Sa roughness from 0.27 (0.10) µm to 0.32 (0.13) µm, 0.20 (0.08) µm to 0.24 (0.09) µm, 0.26 (0.12) µm to 0.33 (0.10) µm and 0.20 (0.07) µm to 0.25 (0.10) µm respectively ($p>0.05$).

Table 16: Mean (SD) Sa roughness of natural unpolished enamel sample before and after *ex vivo* and *in vivo* acid challenges 3x 5 min, 3x 10 min and 3x 15 min. ^{NS}=P>0.05

Group	Sa Before (µm)	Sa After (µm)
1. 3x 5 min *ex vivo*	0.31 (0.14)	0.23 (0.08) ^{NS}
2. 3x 10 min *ex vivo*	0.27 (0.10)	0.32 (0.13) ^{NS}
3. 3x 15 min *ex vivo*	0.20 (0.08)	0.24 (0.09) ^{NS}
4. 3x 5 min *in vivo*	0.26 (0.12)	0.33 (0.10) ^{NS}
5. 3x 10 min *in vivo*	0.25 (0.14)	0.18 (0.08) ^{NS}
6. 3x 15 min *in vivo*	0.20 (0.07)	0.25 (0.10) ^{NS}

Polished enamel significantly increased in mean (SD) Sa roughness for each erosion group. Group 1 (3x 5 minutes erosion *ex vivo*) significantly increased from 0.04 (0.01) µm to 0.09 (0.03) µm ($p<0.05$). Group 2 (3x 10 minutes erosion *ex vivo*) significantly increased from 0.04 (0.01) µm to 0.12 (0.04) µm ($p<0.05$). Group 3 (3x 15 minutes erosion *ex vivo*) significantly increased from 0.04 (0.01) µm to 0.13 (0.04) µm ($p<0.05$). Group 4 (3x 5 minutes erosion *in vivo*) significantly increased from 0.04 (0.02) µm to 0.08 (0.04 µm) ($p<0.05$). Group 5 (3x 10 minutes erosion *in vivo*) significantly increased from 0.04 (0.01) µm to 0.10 (0.04) µm ($p<0.05$). Group 6 (3x 15 minutes erosion *in vivo*) significantly increased from 0.04 (0.01) µm to 0.07 (0.03) µm ($p<0.05$). Therefore, for the rest of the thesis polished roughness results will be discussed as roughness change. Roughness change for Groups 1 to 6 was 0.06 (0.03) µm, 0.08 (0.05) µm, 0.09 (0.05) µm, 0.04 (0.03) µm and 0.06 (0.04) µm 0.04 (0.03) µm respectively and are shown in Table 17 and Figure 53 below. There were statistical differences in roughness change between groups 3 and 6 only ($p< 0.05$).

The mean (SD) step height for polished surfaces in groups 1 to 6 were 2.26 (1.23) µm, 2.42 (1.07) µm, 3.6 (2.68) µm 2.16 (2.5) µm, 2.62 (2.03) µm and 2.43 (2.62) µm respectively. There were no statistical differences between the groups ($p>0.05$) as shown in Table 17 and Figure 54.

The mean (SD) microhardness significantly decreased for all groups. Group 1 (3x 5 minutes erosion *ex vivo*) significantly decreased from 332.8 (41.1) KHN to 132.25 (39.2) KHN ($p<0.05$). Group 2 (3x 10 minutes erosion *ex vivo*) significantly decreased from 326.4 (23.7) KHN to 88.4 (21.1) KHN ($p<0.05$). Group 3 (3x 15 minutes erosion *ex vivo*) significantly decreased from 340.6 (21.2) KHN to 67.6 (14.2) KHN ($p<0.05$). Group 4 (3x 5 minutes erosion *in vivo*) significantly decreased from 318.0 (18.0) KHN to 176.0 (43.5) KHN ($p<0.05$). Group 5 (3x 10 minutes erosion *in vivo*) significantly decreased from 327.1 (21.8) KHN to 136.1 (74.0) KHN ($p<0.05$). Group 6 (3x 15 minutes erosion *in vivo*) significantly decreased from 334.7 (20.8) KHN to 167.7 (55.6) KHN ($p<0.05$). Therefore, for the rest of the thesis polished microhardness results will be discussed as microhardness change (i.e. reduction). Microhardness change for groups 1 to 6 was 190.9 (59) KHN, 241.9 (27.9) KHN, 246.9 (36.9) KHN, 159.6 (66.1) KHN, 190.8 (82.1) KHN and 168.2 (78.2) KHN respectively and are shown in Table 17 and Figure 55 below. There were statistical differences in microhardness change between groups 2 vs. 4 ($p<0.05$), 3 vs. 4 ($p<0.01$), 3 vs. 6 ($p<0.01$).

Table 17: Mean (SD) Sa roughness change, Microhardness change and Step Height for polished enamel samples after *ex vivo* and *in vivo* acid challenges 3x 5 min, 3x 10 min and 3x 15 min.

Group	Mean (SD) **Sa roughness change (µm)**	Mean (SD) **Microhardness Change (KHN)**	Mean (SD) **Step height (µm)**
1. 3x 5 min *ex vivo*	0.06 (0.03)	190.9 (59)	2.26 (1.23)
2. 3x 10 min *ex vivo*	0.08 (0.05)	241.9 (27.9)	2.42 (1.07)
3. 3x 15 min *ex vivo*	0.09 (0.05)	246.9 (36.9)	3.6 (2.68)
4. 3x 5 min *in vivo*	0.04 (0.03)	159.6 (66.1)	2.16 (2.5)
5. 3x 10 min *in vivo*	0.06 (0.04)	190.8 (82.1)	2.62 (2.03)
6. 3x 15 min *in vivo*	0.04 (0.03)	168.2 (78.2)	2.43 (2.62)

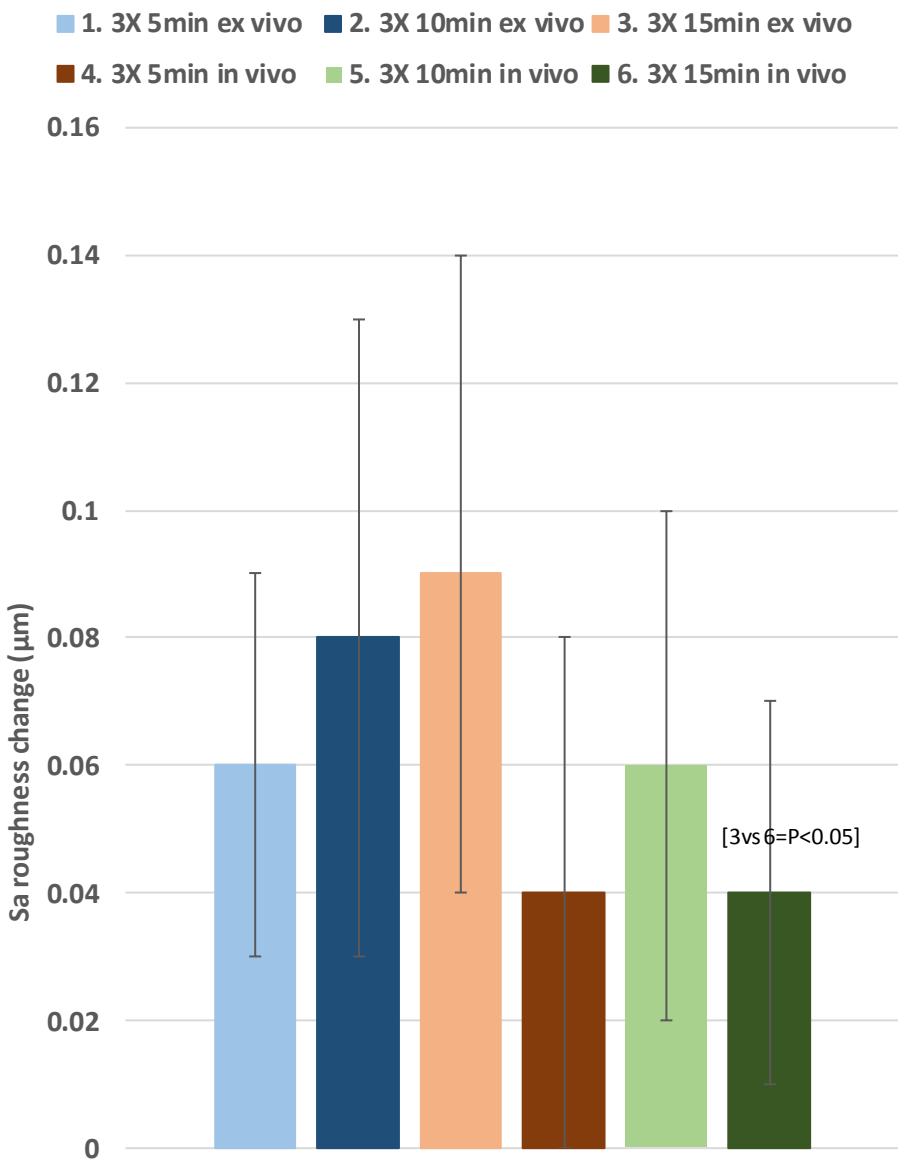


Figure 53: Mean (SD) Sa roughness change of polished enamel following ex vivo and in vivo acid challenges 3x 5 min, 3x 10 min and 3x 15 min. There was significant difference between 3x 15 min ex vivo vs. 3x 15 min in vivo. *=P<0.05

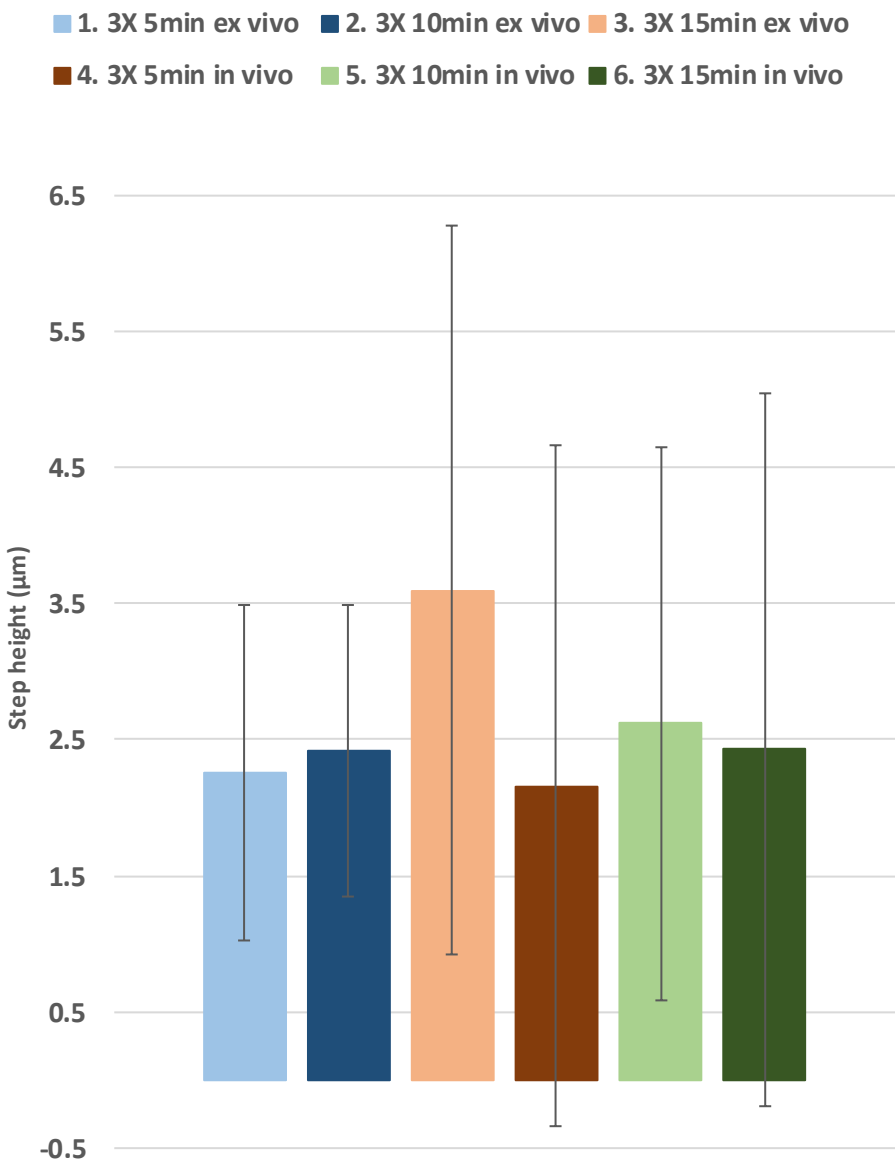


Figure 54: Mean (SD) step height of polished enamel following *ex vivo* and *in vivo* acid challenges 3x 5 min, 3x 10 min and 3x 15 min. There were no statistically significant differences P>0.05.

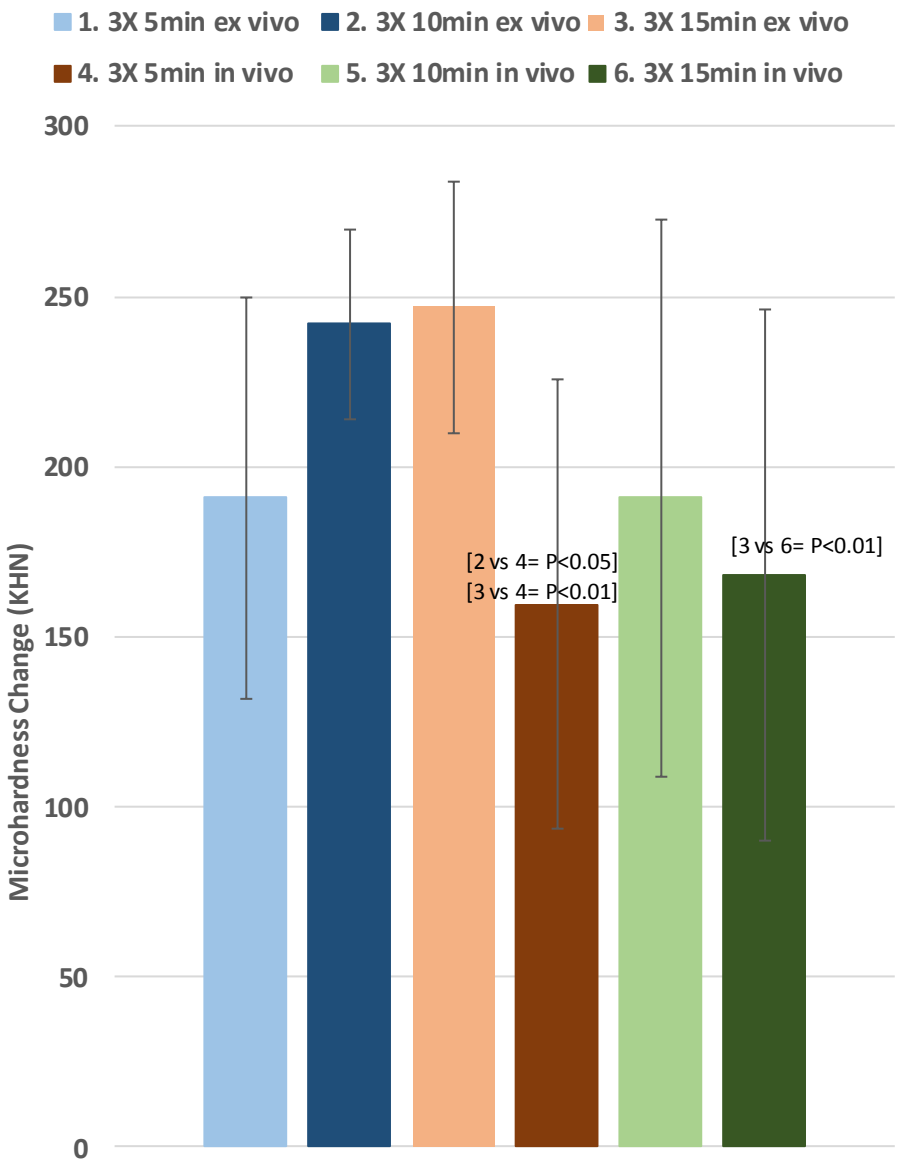


Figure 55: Mean (SD) microhardness change of polished enamel following ex vivo and in vivo acid challenges 3x 5 min, 3x 10 min and 3x 15 min. There were significant differences between 3x 15 min ex vivo vs. 3x 15 min in vivo P<0.01, 3x15 min ex vivo vs. 3x 5 min in vivo P<0.01 and 3x10 min ex vivo vs. 3x 5 min in vivo.

6.6 Discussion

The earlier chapters in this thesis demonstrated how a method was developed to quantify surface of roughness of natural unpolished and polished enamel to be used an *in situ* clinical trial. The clinical trial in this present chapter included the influence of the salivary pellicle and saliva flow (*in vivo* vs *ex vivo* erosion) which provided a closer representation of the clinical scenario. Whilst natural unpolished

enamel became significantly smoother after 45 minutes of erosion *in vitro*, the natural unpolished enamel in the *in situ* study exhibited no statistically significant changes in Sa roughness after either 15, 30 or 45 minutes *in vivo* or *ex vivo* erosion in the same orange juice. Furthermore, there were no identifiable trends in surface alteration of natural unpolished enamel, suggesting that the biological variables mentioned above had a significant influence in modifying the rate of progression of the erosion. Simulating *in vivo* erosion is restrictive as there must be a balance between enough erosion to identify changes but not cause irreversible harm to the participants. Therefore, the erosion regime used *in situ* may have been insufficient to identify changes due the natural protective effects of saliva.

During the *in situ* study two participants reported sensitivity the next day after completing the study. Following topical application of fluoride varnish and sealant the issue fully resolved within 24 hours. However, in relation to this, the daily acid challenge should not be increased beyond 45 minutes. Previous studies have used repeated acid challenges over consecutive days, this may reduce participant compliance but would allow for investigation of the cumulative effect investigating repeated values days which not possible in this study (Hughes et al. 1999b; West et al. 1998; Hooper et al. 2004). This would allow for the compromise needed to detect changes in both polished and natural unpolished enamel.

Polished enamel exhibited significant increases in Sa roughness, step height loss and significant decreases in microhardness following 15, 30 or 45 minutes *in vivo* or *ex vivo* erosion. This reaffirms conclusions in Chapter 5 which identified that natural unpolished enamel was more resistant to erosion than polished enamel. When *in situ* is considered the effects of both the salivary pellicle and saliva flow further increase the resistance of natural unpolished enamel.

The only data which was non-normally distributed was the step height data. This was Log transformed to become normally distributed as it is suggested that Log Transformed data should be preferred to non-parametric analysis (Keene 1995). The data was expressed as mean (SD) in the results section to

improve clarity, and this follows the convention used by other authors (Attin et al., 2003; Ranjitkar et al., 2009).

The inability to identify trends in Sa roughness of natural unpolished enamel may be explained by the effects of the pellicle and saliva on the surfaces themselves, which were not investigated before erosion and no previous studies have been carried out using natural unpolished enamel. To ensure no residual pellicle was left behind and skew the measurements all samples were ultrasonicated in sodium hypochlorite for 30 minutes (Hannig et al. 2005). However, when polished enamel is immersed in saliva post erosion it becomes significantly smoother (Austin et al. 2016). From the results in this thesis, this may not be the case for natural unpolished enamel where interaction between the surface and saliva creates a more complex network. This negative finding also has other implications. It remains uncertain why after 45 minutes there was no detectable change to natural unpolished enamel following this prolonged immersion time. Other laboratory and clinical studies imply that frequent consumption of acids increase the risk of erosion, whereas in this study 45 minutes resulted in no obvious change (Featherstone & Lussi 2006; Willershausen et al. 2008; O'Toole et al. 2017). Perhaps surface roughness does not effectively measure change, or perhaps other factors act upon enamel to cause erosion. Either way this prolonged immersion time with relatively little complications may serve future experimental models.

There were some limitations within the study which may have had an impact on the results with regards to the study population. The participants were recruited depending upon their suitability with regards to the inclusion/exclusion criteria which resulted in a female to male ratio of 2:1. The prevalence of erosive tooth wear is more dominant in males compared to females which may be related to an innate susceptibility (Van't Spijker et al. 2009). Uhlen et al. (2016) investigated whether the susceptibility to erosion amongst different individuals related to the teeth or the overall oral environment. They identified that the overall oral environment had the biggest influence. Therefore, in an attempt to reduce the variability future studies could use either all male or all female

participants. The saliva flow rate of the participants was not assessed for their recruitment into the study. Reduced salivary flow results in less acid clearance (Buzalaf et al. 2012). There is a general consensus between studies identifying lower unstimulated salivary flow rates in people with erosive tooth wear compared to those without. Therefore, differences in variation of this could also have increased variability.

From the early pilot work outlined in Chapter 2 it had been determined that microhardness and step height measurement would not be possible with the natural unpolished enamel samples. Therefore, surface roughness measurements were the sole quantitative analysis of natural unpolished enamel throughout this thesis. Mineral analysis could have complemented this data but was not possible due to logistical reasons.

However, the *in situ* study complemented the earlier work by allowing investigation into the effect of the salivary pellicle and salivary flow. Saliva provides protection from formation of the salivary pellide. To allow for pellicle formation the participants wore the intra-oral appliance for 30 minutes prior to the first acid challenge and subsequently for 1 hour between the next acid challenges. Saliva also has a protective effect through physiological means, washing away debris and buffering acidic solutions. The *ex vivo* acid challenges allowed investigation into the effect of pellicle formation, whilst the *in vivo* challenges allowed investigation into the full physiological effect of saliva. The Sa roughness results of polished enamel from *ex vivo* acid challenge demonstrated an increase in roughness change with increase in erosion duration, 0.06 (0.03) µm after 15 minutes, 0.08 (0.05) µm after 30 minutes and 0.09 (0.05) µm after 45 minutes. These indicate that the erosive effects on surface roughness change of polished enamel were approximately one third of those seen when erosion was carried out *in vitro* without the presence of the salivary pellicle demonstrating its protective effect. Nekrashevych and Stösser (2003) previously suggested that the protective effect of the salivary pellicle lasted for 10 minutes. This is supported by the findings in this study as immersion for only 15 minutes demonstrated a significant increase in Sa roughness, a significant decrease in microhardness and measurable step

height loss of polished enamel. The mean (SD) step height measurements after immersion in orange juice have similarities to other studies. Moazzez et al. (2014) identified a step height loss of 1.34 (0.66) µm on pellicle coated polished bovine enamel samples following *ex vivo* immersion in pH 3.2 citric acid for 10 minutes. Whilst these trends were identified there were no statistical differences among the groups. The cyclic nature of erosion regime allowed for pellicle to reform between rinses therefore, each acid challenge would begin with 10 minutes pellicle protection minimising the effects and making it more difficult to differentiate between the groups.

However, the polished samples in the *in vivo* acid challenge groups did not demonstrate clear trends. Sa roughness change after 15, 30 and 45 minutes acid challenge was 0.04 (0.03) µm, 0.06 (0.04) µm and 0.04 (0.03) µm respectively. Fully understanding the changes in Sa roughness is challenging. Sa roughness measurements are calculated from height deviations within the form of surface, Ra from a single line of a surface and Sa from the overall surface. Increased deviations result in rougher surfaces and less deviations, smoother surfaces (Field et al. 2013). However, average amplitude parameters like Sa and Ra are unable to distinguish between peaks and pits, and deviations from peaks can sometimes result in the same average value (Ra or Sa) as deviations from pits as shown in Figure 56 below (Leach 2014). This has been identified in dentine abrasion. Mullan et al. (2017a) identified significant increases in Sa roughness of polished dentine samples that underwent erosion-abrasion with a standard fluoride toothpaste and samples that underwent erosion-abrasion using a desensitising toothpaste. Despite both groups increasing in Sa roughness the standard toothpaste group experienced increase in tubule patency whereas tubule occlusion occurred with the desensitising toothpaste.

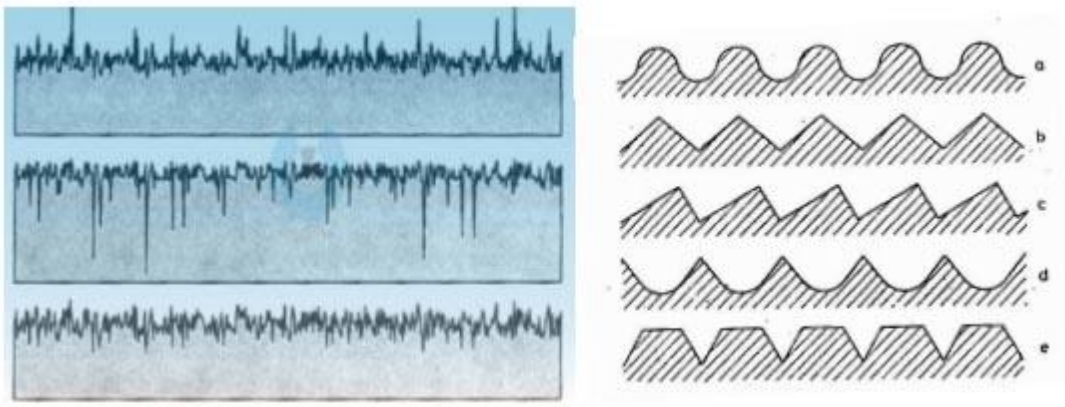


Figure 56: Demonstration of how one Ra value can be calculated from different shapes (CNCCookbook 2017).

However, in this current study the differences in Sa roughness are likely to be a true effect where the samples are truly becoming smoother at the increased erosion time (45 minutes). The increased time allowed for increased washing and buffering effect with the increased salivary flow, particularly as unstimulated salivary flow rate and buffering capacity have been directly associated with dental erosion (Zero & Lussi 2005). Austin et al. (2016) investigated the remineralising effect of saliva on Sa roughness by immersing eroded polished enamel in saliva. They identified that whilst the acid challenge resulted in significant increases in roughness, following immersion in saliva there were significant decreases in roughness. Therefore, the saliva flow may be reducing the roughness of the samples which when calculating roughness change from baseline and after completion of the 3-cyde acid challenges totalling 45 minutes erosion it appears there has been less roughness change. However, what is more likely is that within the acid challenges there has been a combination of roughness increases and decreases. This would result in the same effects seen in microhardness change and explain why 45 minutes erosion *in vivo* resulted in significantly less roughness and microhardness change compared with 45 minutes erosion *ex vivo.* However, the relationship between microhardness change and the effect of saliva is interesting. In an *in vitro* study comparing the effects of distilled water, artificial saliva and natural saliva on microhardness change of enamel samples it was

identified that the enamel samples immersed in natural saliva become significantly softer (Mutahar et al. 2017). These findings correspond with the *ex vivo* results in this Chapter, however where *in vivo* immersion is considered the relationship is more complex and future work is needed to explore this relationship further. Step height measurements may not have identified significant differences between the groups but revealed a similar pattern with increased step height loss from 15 to 30 minutes *ex vivo* acid challenge and then a reduction from 30 to 45 minutes.

6.7 Conclusions

The null hypothesis can be rejected for polished enamel which exhibited significant increases in Sa roughness and microhardness change following 15, 30 or 45 minutes erosion in dietary acid *in situ*. However, the null hypothesis is retained for natural unpolished enamel.

Final conclusions

This thesis developed a method to investigate surface roughness changes of natural unpolished enamel and polished enamel following dietary erosion. At the beginning of this thesis there were few studies which used natural unpolished enamel as a substrate. Therefore, the early pilot work were novel findings, and as the topic is of interest to other researchers there have been publications which have validated these early findings. The *in situ* aspect of this thesis, with the *in vivo* and *ex vivo* immersion, remains novel and complemented the early *in vitro* work. Overall, from *in vitro* and *in situ* investigation of increasing immersion times the extent of erosion required to exhibit Sa roughness change of natural unpolished enamel was significantly longer than that required for polished enamel and once outer enamel had been removed there was likely to be a much lower threshold for continued progression of erosive wear. For changes in surface roughness to be detected in natural unpolished enamel structural breakdown has also had to occur. Therefore, the exact nature of the first sign of erosive tooth wear 'initial surface texture loss' still remains to be defined.

Relevance for clinical practice

Erosive tooth wear is a prevalent oral disease with epidemiological evidence of both increasing prevalence and pathogenesis (Lussi & Carvalho 2014). Tooth wear has evolved from being considered a natural benign process to be recognised as a pathological disease. Pain, loss of function, serious aesthetic deterioration and a balance between whether 'the tooth will survive the rate of wear' change tooth wear from a physiological to pathological condition (Smith & Knight 1984). There has been increased interest in research involving erosive tooth wear (Lussi and Carvalho 2014). However, despite this increased research and awareness the prevalence of erosive tooth wear is still increasing (Van'T Spijker et al. 2009; Kreulen et al. 2010; White et al. 2012). Therefore, there remains much more to be done particularly for the early detection and prevention of erosive tooth wear. Most clinical case reports refer to generalised tooth wear, however generalised severe erosive tooth wear would actually be a more fitting description. The fact remains that patients are still presenting after severe irreversible tooth surface loss has occurred, often requiring complex multidisciplinary intervention (Muts et al. 2014; Song et al. 2010; Leung et al. 2016). Therefore, there is a deficit for clinical detection and monitoring of early erosive tooth wear. Indices such as the BEWE can be unreliable for general practice as they rely on visual assessment which will rely upon the examiner's perception. It is not standardised amongst dentists and even consistency of an individual dentist can vary. Furthermore, the initial sign of erosive tooth wear 'early surface texture loss' still remains undefined and perhaps even visually undetectable (Bartlett 2016). Few of the laboratory techniques that researchers use can be adapted for clinical use. Superimposition techniques to quantify tissue loss (profile measurements) over a period of time can be used *in vivo* (Rodriguez et al. 2012b). However, these are limited due to the length of time required for adequate tissue loss to be detected, which one could argue is allowing for irreversible loss to occur in this time and could therefore be considered unethical. Moreover, the intention is to detect the earliest indications of erosive tooth wear before irreversible damage has occurred. Quantitative light-induced fluorescence (QLF) has been successfully used to detect caries *in*

vivo and has been considered for the detection of early erosive tooth wear. However, preliminary work revealed only a weak correlation between QLF and gold standard indicator of erosive tooth wear Transverse Microradiography (TMR) (Elton et al. 2009). However, TMR can overestimate lesion depths as it can include regions of subsurface demineralisation which are not the true lesion depth. NCP measures to the true base of the lesion and a recent *in situ* demonstrated promising trends between step height (measured by a NCP) and QLF analysis (Ablal et al. 2017). This remains a promising area for future development. Optical coherence tomography (OCT) has also been advocated for *in vivo* use for its ability to discriminate between sound and demineralised enamel, but limitations include the need for reference areas of non-affected enamel that must be adjacent for comparison. This would mean covering part of a person's tooth *in vivo,* plus reference markers would be required to ensure the same location is examined at subsequent visits and previous work has shown these to be ineffective *in vivo* (Sundaram et al. 2007). There have been rapidly evolving advances in the use of surface texture measurements such as surface roughness (Austin et al. 2015).

Surface roughness changes are advocated for initial changes before bulk tissue loss has occurred and therefore, suggesting they could detect tissue changes from erosion that are still reversible. There are complexities when measuring surface texture of curved and complex structured surfaces such as human enamel. This thesis developed a model to quantify the surface texture of natural unpolished enamel and identify changes which occurred following initial erosion by dietary acid. It was identified that *in vitro* natural unpolished enamel was naturally more resistant to erosion than laboratory process polished enamel and that it behaved differently, becoming smoother after erosion mimicking the clinical description 'smooth and shiny' (Amaechi & Higham 2005). Furthermore, it was identified that although 45 minutes erosion in orange juice was sufficient *in vitro* to induce significant changes in the surface texture of natural unpolished enamel, when an *in situ* model was applied to investigated the effects of the pellicle and saliva any textural changes were inhibited. Thus, demonstrating the innate resistance of natural teeth to erosive tooth wear. Further developments in the erosion model

used would be required to break through this innate resistance. This is clinically relevant as population studies are querying the timing and frequency as well as the quantity of acidic products ingested in relation to the prevalence of erosive tooth wear (O'Toole et al. 2017). However, the residing measurement uncertainty with surface roughness measurements means that other techniques need to be reconsidered.

Further work

Overall Sa roughness did not prove to be a successful predictor of early signs of erosive tooth wear. Whilst, some authors have suggested that other parameters to quantify surface roughness may identify changes undetected by Sa roughness their use *in vivo* would be limited. Hybrid techniques such as area scale fractal analysis (Asfc) are site dependent and do not provide an overview of an overall surface which Sa provided (Leach et al. 2008). Therefore, other methods to identify the early signs of erosive tooth wear *in vivo* must be investigated.

- Surface reflectometry has been suggested for *in vivo* application (Carvalho et al. 2016b). A hand held reflectometer which can be used chairside has been developed and early studies show a correlation between increased diffuse reflectometry, decreased specular spectrometry and initial signs of erosive tooth wear (Rakhmatullina et al. 2011; Rakhmatullina et al. 2013; Carvalho et al. 2016b).
- Optical Conference Tomography (OCT) has also been suggested to be capable of identifying erosive tooth wear *in vivo.* OCT is a non-invasive technique, similar to an ultrasound and can detect changes in both surface texture and depth (Joshi et al. 2016). There has been increased interest in its use for *in vivo* studies (Field et al. 2010; Austin et al. 2017). A recent *in vitro* study which investigated different wear mechanisms using natural unpolished enamel differentiated between the different wear patterns with OCT imaging (Mercuţ et al. 2017). However, further, work involving OCT and clinical studies is needed.

- Further work using replica techniques and profile measurements to determine how much time is required for measurable loss to occur. Previous work was unable to identify measurable loss (as the tissue loss measured was less than the measurement error of the equipment) (Rodriguez et al. 2012b). However, a prolonged longitudinal or *in situ* study model could be implemented to investigate this further.

IOP Publishing Surf. Topogr.: Metrol. Prop. 3 (2015) 023002 doi:10.1088/2051-672X/3/2/023002

Surface Topography: Metrology and Properties

TOPICAL REVIEW

Surface texture measurement for dental wear applications

RECEIVED
22 December 2014

REVISED
25 February 2015

ACCEPTED FOR PUBLICATION
3 March 2015

PUBLISHED
2 April 2015

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Keywords: dental enamel, surface texture, surface metrology

Abstract

The application of surface topography measurement and characterization within dental materials science is highly active and rapidly developing, in line with many modern industries. Surface measurement and structuring is used extensively within oral and dental science to optimize the optical, tribological and biological performance of natural and biomimetic dental materials. Although there has historically been little standardization in the use and reporting of surface metrology instrumentation and software, the dental industry is beginning to adopt modern areal measurement and characterization techniques, especially as the dental industry is increasingly adopting digital impressioning techniques in order to leverage CAD/CAM technologies for the design and construction of dental restorations. As dental treatment becomes increasingly digitized and reliant on advanced technologies such as dental implants, wider adoption of standardized surface topography and characterization techniques will become evermore essential. The dental research community welcomes the advances that are being made in surface topography measurement science towards realizing this ultimate goal.

1. Introduction

Following the myriad developments by advanced manufacturing industries in enhancing material properties through 'structuring' or 'texturing' surfaces (Evans and Bryan 1999); the optimization, enhanced characterization and measurement of the surface features of dental hard tissues and dental biomaterials has driven some of the most important discoveries in oral and dental science to date.

The dental use of acid etching solutions and airborne-particle abrasion are relevant for a number of clinical applications routinely used today. Since the mid-1950s, intentional roughening of enamel and dentine with the aim of providing adhesive bonding of resin-based dental biomaterials to intraoral structures has been ubiquitous in clinical dental practice (Buonocore 1955). With regard to dental biomaterials, the foremost example of dental surface engineering, involves the surface roughening of commercially pure titanium implants to optimize the biological phenomenon coined 'osseointegration' (Branemark 1983), which currently underpins the safe and predicable replacement of millions of missing teeth using dental implants. Acid-etched enamel and rough-surface dental implants are but two examples of bio-technologies which are reliant on surface texture mediated phenomena, the predicable application of which is dependent on our understanding of how natural and biomimetic surfaces function within hostile biological environments.

This paper will review pertinent examples of how the successful application of surface topography and texture engineering, metrology and characterization have enabled the widespread adoption of what we currently understand as state-of-the-art applied clinical technologies in oral and dental science. The paper will also review the limitations of the dental industry's use and understanding of surface topography measurement science and suggest how the enhanced uptake of recent developments in the standardization of measurement of surface form and texture can further benefit research and clinical practice in oral and dental science.

2. Surface topography measurement of natural dental hard tissues (enamel and dentine)

The main clinical surface event with relevance to surface topography metrology and characterization at both the micro and the macro scale is dental wear

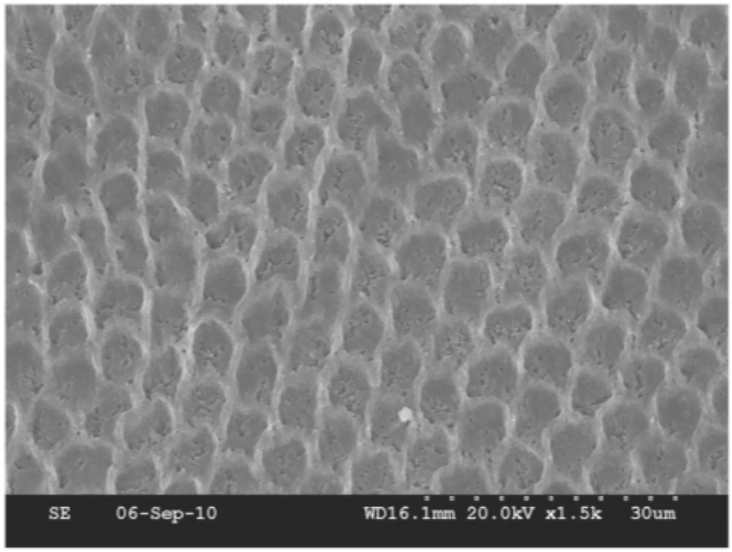

Figure 1. Scanning electron micrography image of exposed enamel prisms in a polished human enamel sample.

which clinically involves a combination of acidic chemical wear (from acidic foods, drinks, gastric juices and industrial origins) and mechanical wear.

Human dental enamel has evolved as a exquisitely hierarchically structured tissue composed mostly of hydroxyapatite crystals (85% volume) interspersed with water (12% volume) and proteins and lipids (3% volume) (Nanci and Cate 2008). At the microstructural level, the basic feature is the enamel rod or prism which has a diameter of between 4 μm and 6 μm and decussates at the enamel surface (Berkovitz 2009). The interprismatic enamel has greater intercrystalline space, which as illustrated in figure 1 above the density of prisms and their mineral content is not uniform across the enamel surface, which in turn fundamentally affects the surface dimensionality of common physiological and pathological events occurring to the microscale enamel surface in the oral environment.

At a larger scale, as shown in figure 2 below these 4–6 μm enamel prismatic textural features are superimposed on longer range features, which represent growth lines (perikymata) formed in the enamel surface during amelogenesis which occurs prior to the tooth erupting into function.

2.1. Application of surface topography metrology to dental wear of human enamel

The dental wear terminology differs somewhat from the tribological literature in that the chemically mediated processes which initiate dental enamel wear are known as dental erosion (Bartlett 2005), rather than 'corrosion' as it may be know in tribology (Zhou *et al* 2013). Dental erosion is a demineralizing surface phenomena involving enamel exposure to acidic solutions deriving from either dietary acids or regurgitated stomach acids (Larsen 1973, West and Joiner 2014). Subsequently, 'attrition' is dental wear from mechanical tooth-to-tooth contact and 'abrasion' is mechanical dental wear from other objects such as coarse foods, tooth brushing and other oral habits (Addy and Shellis 2006). Collectively these chemical and mechanical processes act synergistically to result in a clinical disease known as erosive tooth wear. At the histological level the textural changes caused by erosion are shown in figures 1 and 2 above.

Clinically, the initial presenting signs that a dental care professional is looking for during a dental examination are described clinically as 'initial loss of surface texture' (Bartlett *et al* 2008). These initial signs can be seen when comparing the photographs seen in figures 3 and 4 below. The clinical finding of 'initial loss of surface texture' refers to changes to the natural primary, secondary and tertiary surface anatomy seen in healthy teeth when they erupt in their virgin state in comparison to eroded and worn teeth such as seen in figure 4 and figure 5 below.

Tooth wear is a widely prevalent clinical problem, which has affected all dental populations over the ages. From an anthropological perspective the main cause has been attributed to the coarse diet of prehistoric humans and therefore this was considered to be a part of normal physiological processes (Kaidonis 2008). However, with the advent of the modern diet coupled with the increased longevity and general health

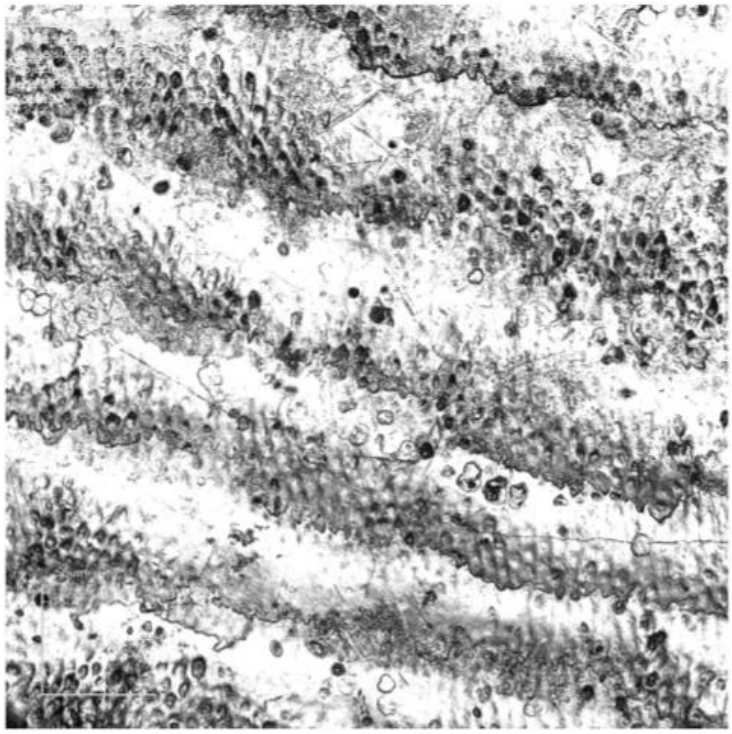

Figure 2. Confocal laser scanning micrograph of the curved outer enamel surface of a human molar after exposure to orange juice showing the acid exposure of enamel prisms in a banding pattern across the perikymata (growth lines formed during amelogenesis).

Figure 3. Photograph of a healthy dentition with no erosive tooth wear and sound enamel.

improvements of modern mankind, there is a huge increase in the retention of all natural teeth which correspondingly has increased the focus on protecting enamel from the earliest signs of wear (Steele *et al* 2012). Due to increased consumption of acidic foods and beverages, tooth wear is becoming increasingly prevalent with currently over 70% of the dentate UK population showing some signs of tooth wear (Steele and O'Sullivan 2011).

At both the macroscopic and the microscopic levels there has been significant research to employ surface metrology techniques to measure and characterize the histological and clinical features of tooth wear. The exact geometry of the macroscopic clinical

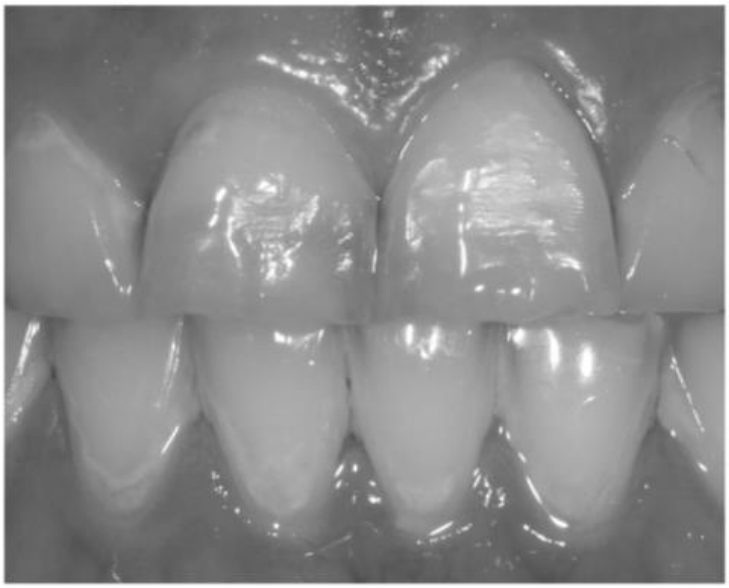

Figure 4. Photograph of central and lateral incisors showing initial loss of surface texture.

features broadly classified in table 1 above often depend upon the main predisposing element of the tooth wear but all result in changes to the normal anatomy of the cusps (peaks) and fossae (valleys) of the tooth as it is in its virgin state when freshly erupted. Attrition is characterized by flattened occlusal (biting) surfaces of the teeth and wear facets in teeth often have a smooth and shiny appearance. Erosion causes a generalized silky appearance to the tooth surface, often exposing the underlying dentine in isolated pits. The overall result is a combination of the shiny smooth surfaces, cupped occlusal surfaces and wedge shaped facets, as seen in figure 5 below.

As clinical scoring systems are qualitative methods of assessment there have been many attempts at using more quantitative methods to assess the changes on the enamel surface, mostly focusing on macroscale profile measurements of stone cast replicas of the teeth or at the micro and nanoscale textural level using extracted teeth subjected to simulated tooth wear in the laboratory. The measurement instrumentation employed for this includes contacting and non-contacting surface profile and texture instruments (both line profiling and areal topography characterization) (Schlueter *et al* 2011).

A recent study comparing the use of contacting profilometry, non-contacting profilometry and confocal laser scanning microscopy in a dental erosion laboratory study found that the three instruments produced comparable results and were effective in the assessment of erosion using a step height measurement technique (Paepegaey *et al* 2013). However, the potential benefits of confocal laser scanning microscopy in comparison to the other techniques are the smaller laser spot size resulting in improved lateral resolution and thus potentially higher quality measurement for the key features of the 4–6 μm enamel prism (Leach 2011). In other studies similar instruments have been used to assess bulk tissue loss (step height and volume) (Austin *et al* 2010, Rodriguez *et al* 2012) as well as 2D and 3D surface texture parameters (Schlueter *et al* 2011).

In terms of surface texture parameters, although a review of dental erosion studies shows that historically Ra has been the most commonly cited parameter (Field *et al* 2010), more recently there have been attempts to adopt 3D areal texture parameters (Leach 2011). In recent laboratory studies areal surface texture analysis in combination with confocal laser scanning microscopy has proved a very sensitive techniques for characterization of early erosion (Mann *et al* 2014), which demonstrates all the inherent benefits of 3D surface texture characterization of a structured surfaces such as dental enamel (Leach 2013) and will, in time, enhance understanding of the fundamental mechanisms underpinning the wear process.

Figure 5. Photograph of lower first permanent molar with a enamel and dentine tissue loss ⩾50% of the surface area of the tooth.

2.2. Anthropological surface metrology for dental microwear analysis

The application of dental microwear analysis of fossilized teeth has been used within the anthropological community, largely qualitatively, in order to attempt to provide direct evidence of what an prehistoric individual ate during their lifetime (Walker *et al* 1978). The form of wear facets and gradient of wear along the dentition has shown contrasts, particularly between hunter-gatherers and agriculturalists (Hillson 1996). Surface texture measurement and characterization of wear faceting of excavated teeth has revealed patterns of scratches and pits in the enamel surface and a large body of work has investigated these patterns in order to try to understand their relationship with the nature of the diet. This has often employed unstandardized analytical techniques which relied on small sample sizes, subjective identification of individual surface features and observer error (Grine *et al* 2002).

More recently, laser confocal scanning microscopy together with scale-sensitive fractal analysis has been used to characterize the complexity and anisotropy of microwear in hominin fossils (Scott *et al* 2005) which has resulted in a greater potential for reconstructing the diets of extinct hominins with greater repeatability and reduced observer error. This demonstrates another example of the dental research community adopting good practice measurement techniques from developments in metrological research standards and techniques.

3. Surface topography measurement of dental biomaterials

As stated above, the oral environment is chemically and mechanically hostile to even the best designed biological materials such dental enamel, which has evolved excellent toughness and durability over millennia, in order to resist the surface challenges from

Table 1. The most commonly used methods for 2D and 3D quantitative analysis of dental surfaces and biomaterials.

Quantitative analysis method	Cited parameter	Applications in oral and dental science	Advantages	Disadvantages
Laboratory based contacting instrumentation	2D texture parameters (Sa, Sq) bearing parameters based on the Abbot-Firestone curve	Quantifying roughness of filling materials and dental hard tissues during dental wear research quantifying bulk loss of dental hard tissues	Simple and inexpensive technique	Ra contains no information about the textural characteristics of a profile, the likelihood of future wear or wear-resistance, the rate of future wear or the potential of a surface to retain fluids/lubricants.
Laboratory based optical instrumentation	2D and 3D texture parameters including amplitude, functional and hybrid 3D parameters	Anthropological dental research dental wear research	Areal surface texture analysis can characterize a wider range of information from a surface (or a replica thereof), including relative areal-scale analysis and other advanced texture techniques	Not widely used in dental research perhaps due to limitation of access to advanced instrumentation and skills mostly cannot be used for clinical measurements of surface texture *in vivo*
Clinical intra-oral dental scanners	3D surface profile data (ASCII or. stl file formats)	Clinical construction of dental prostheses using CAD–CAM technologies	Can directly capture intra-oral information of dental hard and soft tissue thus avoiding use of conventional dental impressions	Many require optically active powders to facilitate stitching across the dental arch and currently no reliable surface texture measurements can be taken

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day-to-day functioning (Zhou *et al* 2013). The field of dental materials science therefore relies heavily on the ability to measure and characterize the functional surface properties of dental biomaterials. These include optical, tribological, and biological properties of biomimetic materials including functional and aesthetic restorative (filling) materials, ceramics, titanium dental implants, dental adhesives and abrasives, denture-base acrylics or metal alloys and daily oral care products such as toothpastes and tooth whitening gels which are used daily by millions of people across the globe. All of these biomaterials have been developed with cogniscence of how they interact on a surface level with the oral hard and soft tissues, as well as the ubiquitous oral microbiota, including biofilms, bacteria, fungi and viruses.

3.1. Surface properties of restorative dental materials and dental abrasives using to preserve diseased and damaged teeth

Controlling the surface texture of restorative dental materials such as tooth coloured dental filling materials, is essential to successfully restore oral function and aesthetics using biomimetic dental materials. Tongue proprioceptors are able to detect an change in surface roughness (Ra) of 250 nm (Jones *et al* 2004) and for this reason, amongst others, dental materials companies invest enormous resources in developing novel filling materials with optimal surface properties, including through the use of nanotechnology (Kaizer *et al* 2014).

Optimizing the surface texture of dental filing materials also enhances properties such as cleanliness (through reduced plaque biofilm formation occurs on smoother surfaces of the restoration), enhanced aesthetics (though reduced roughness improving surface lustre and gloss) and reduced wear of opposing teeth (smoother surfaces reduce two or three body wear processes) (Heintze *et al* 2006, Heintze *et al* 2012). Hence, the dental industry also continually invests significant research and development resources in bringing to market novel instruments for finishing and polishing dental restorative materials, including fluted carbide burs, diamond burs, pointed stones, coated abrasive strips and discs, polishing pastes, soft and hard polymeric cups, points and wheels impregnated with specific types and sizes of abrasive particles (Anusavice *et al* 2013); all of which are tested in laboratory and clinical conditions mainly using Ra measurements of optically measured surfaces.

These techniques are especially relevant for reducing the abrasiveness of dental ceramics in order to prevent wear of opposing tooth surfaces and to result in improved biomimetics, in terms of the optical gloss and lustre of the ceramic surface. Ideally, the smoothest possible surface of the restorative material should be produced and significant research is ongoing to understand the links between surface roughness and the strength and aesthetics of dental ceramics (Anusavice 1992, Kelly *et al* 1996).

3.2. Surface properties of osseointegrated dental implants used to replace missing teeth

Perhaps the greatest role of surface topography metrology in dental materials science is though 'texturing' of osseointegrated dental implant surfaces. This refers to processes which increase the surface roughness of the implant surface in order to optimize the surface area to which the supporting jaw bone can osseointegrate (Anusavice *et al* 2013).

Osseointegration is the process by which living ordered bone grows to within 100 Å of an inert foreign body (the implant) in order to support a functioning dental prosthesis (crown, bridge or denture), which is used to replace missing teeth (Mavrogenis *et al* 2009). The original commercially pure titanium dental implants were developed in the 1960s and were machined to a relatively smooth surface (Sa $<1.5\ \mu$m) (Newman 2012). Although these early implants successfully oeesointegrated, from the late 1980s onwards, researchers began to find that surface textural modifications of the implant surface enhanced the speed and predictability of the biological process (Parekh *et al* 2012).

Through increased understanding of role of surface topography in osseointegration, surface modifications to increase the surface roughness by coating, blasting by various methods, by acid treatments or by a combination of these treatments were developed and refined. These include ablative procedures such as grit blasting, acid etching, anodizing, shot/laser peening and additive procedures such as plasma spraying, electrophoretic deposition, putter deposition, sol gel coating, pulsed laser deposition and biomimetic deposition. The benefits of the increased surface roughness to osseointegration treatment include improved stability at the time of placement, improved bone to implant contact ratios, increased bone growth (osseoinduction) to the implant surface and increased osteogenic bone cell attachment and growth (Parekh *et al* 2012). Currently all commercially availiable dental implants rely on some form of surface modification to capitalize on the optimal surface roughness and thus more rapid and predicable osseointegration (Cooper 2000).

Over time, clinical research has proven that the that optimal surface topography influences the bone response at the micrometre level with so called moderately rough (Sa 1–2 μm) surfaces show the strongest bone responses due to higher bone to implant ratios (Wennerberg and Albrektsson 2009). However, as with other fields employing surface metrology, the majority of published papers present an inadequate descriptions of surface characterization techniques and researchers in dental implantology are, as with many applied research fields, requiring increased

standardization of measurement and evaluation techniques and a greater adoption and use of spatial and hybrid parameters, such as those being developed in the dimensional metrology fraternity (Leach 2011), in order to continue to progress the field.

4. Discussion

Surface metrology can be used to gain knowledge how the material under investigation was formed; knowledge of the surface events that the material has been subjected to during it's existence so far and finally the ability to predict the likely future behaviour of the surface as it continues to interact with its environment. Dentistry is an excellent example of an applied branch of health care which draws heavily upon this knowledge in order to progress it's underpinning disciplines of biomaterials, biomimetics and biophotonics through which modern treatments and techniques are designed to prevent and treat oral and dental diseases (and their resulting side effects) with increasing sophistication and effectiveness. This review has highlighted a few examples of how this knowledge is gained and utilized within the field of oral and dental science and these findings are summarized in table 1 above, which outlines some of the most commonly used methods for 2D and 3D quantitative analysis of dental surfaces' features.

This review has found that within clinical dentistry and dental biomaterials science there has historically been a wide reliance on the 2D surface texture parameters calculated using relatively simple profiling instrumentation and software parameters. This has resulted in some understanding, for example, of how dental wear processes interact *in vivo* (Azzopardi *et al* 1999, Azzopardi *et al* 2001), however increased use in clinical dental research of the more recently developed areal surface texture characterization techniques will potentially advance our understanding of dental materials surface properties and behaviour (Austin 2013, Mann *et al* 2014a, 2014b). The dental research community is waking up to these advances as dental materials scientists fruitfully collaborate with metrologists who themselves are increasingly using techniques with greater international standardization (Leach 2013).

The need for an enhanced working understanding of surface topography measurement and characterization is becoming increasing essential as dental materials science moves into an increasingly micro and nano-scale world and as the precision engineering industries are increasingly influencing our day to day practice of dentistry. Perhaps the greatest change that is currently occurring within dentistry is the increasing use of advanced digital imaging technologies to prevent and treat every day dental diseases. Technologies that were once only available for a small minority of patients are now becoming ubiquitous largely due to the increased ability of dental industry to design, manufacture and supply these technologies in a cost-effective manner. The uptake of digital impressioning technologies which acquire 3D surface profile images of the teeth and jaws, in order to plan, design and construct dental treatments such as crowns, bridges, implants and orthodontic treatments is driving dentistry away from analogue processes such as conventional dental impressions and casting restorations using lost wax techniques, for example, and towards intra-oral digital imaging and CAD/CAM construction of dental crowns and bridges using CAD software and high precision milling and sintering techniques.

From the perspective of surface topography measurement, digital impressioning technology will increasingly require international adoption of modern surface metrology techniques as the overall performance of these dental restorations will be increasingly determined by surface features such as the surface texture of the manufactured component part rather than it's overall form. Concurrently, enhanced 3D surface data capture of the dental hard and soft tissues using digital dental impressioning will enable the novel diagnostic approaches that until now have relied on the production of stone replicas of the dental hard tissues and scanning under laboratory conditions (Rodriguez *et al* 2012). The ability to capture whole-arch 3D surface data to the micron level requires a greater understanding amongst dental researchers of how to truly define a surface and how to extrapolate and interpret meaningful surface data once a measurement has been made. This ability to perform accurate and precise surface measurement and then to intelligently apply appropriate analytical techniques to the captured data will increasingly rely on meaningful collaboration between dimensional metrologists and dental materials academic and industrial scientists, with the ultimate benefit of improving health care practice, policies and products to optimally treat and prevent oral and dental diseases in the wider population.

5. Conclusion

The application of surface topography measurement and characterization within dental materials science is highly active and rapidly developing, in line with many modern industries. Surface measurement and structuring is used extensively within oral and dental science to optimize the optical, tribological and biological performance of natural and biomimetic dental materials. The review has highlighted that although there has historically been little standardization in the use and reporting of surface metrology instrumentation and software, the dental industry is

beginning to adopt modern areal measurement and characterization techniques. This has relevance for to greater understanding of the fundamental mechanisms underpinning clinical wear of enamel and dentine and clinical research is ongoing to determine if clinical measurement of the surface roughness of dental enamel *in vivo* can be used to detect the earliest signs of dental erosion and therefore prevent its progression.

Another area of ongoing research involves investigating the application of intra-oral digital scanners in dimensional metrology of dental hard tissues, for earlier diagnosis and more accurate monitoring of 3D changes in the mouth related to disease or functional wear. Intra-oral 3D scanners are increasingly being used in clinical practice to replace conventional dental impressions for provision of CAD/CAM dental restorations using 3D software design and milling or printing of a variety of metallic and non-metallic restorations. As modern dental treatment becomes ever more reliant on advanced digital imaging and manufacturing technologies, wider adoption and leverage of internationally standardized surface topography and texture characterization techniques will become increasingly essential. The dental research community welcomes the advances that are being made in surface topography measurement science towards realizing this ultimate goal.

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DENTAL-2941; No. of Pages 9 ARTICLE IN PRESS

DENTAL MATERIALS XXX (2017) XXX.E1–XXX.E9

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Measurement uncertainty associated with chromatic confocal profilometry for 3D surface texture characterization of natural human enamel

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ARTICLE INFO

Article history:
Received 12 December 2016
Received in revised form
13 March 2017
Accepted 10 April 2017
Available online xxx

Keywords:
Surface texture characterisation
Enamel
Erosion
Profilometry

ABSTRACT

Objective. To investigate the measurement performance of a chromatic confocal profilometer for quantification of surface texture of natural human enamel in vitro.

Methods. Contributions to the measurement uncertainty from all potential sources of measurement error using a chromatic confocal profilometer and surface metrology software were quantified using a series of surface metrology calibration artifacts and pre-worn enamel samples. The 3D surface texture analysis protocol was optimized across 0.04 mm^2 of natural and unpolished enamel undergoing dietary acid erosion (pH 3.2, titratable acidity 41.3 mmol OH/L).

Results. Flatness deviations due to the x, y stage mechanical movement were the major contribution to the measurement uncertainty; with maximum Sz flatness errors of 0.49 μm. Whereas measurement noise; non-linearity's in x, y, z and enamel sample dimensional instability contributed minimal errors. The measurement errors were propagated into an uncertainty budget following a Type B uncertainty evaluation in order to calculate the Standard Combined Uncertainty (u_c), which was ±0.28 μm. Statistically significant increases in the median (IQR) roughness (Sa) of the polished samples occurred after 15 (+0.17 (0.13) μm), 30 (+0.12 (0.09) μm) and 45 (+0.18 (0.15) μm) min of erosion ($P < 0.001$ vs. baseline). In contrast, natural unpolished enamel samples revealed a statistically significant decrease in Sa roughness of −0.14 (0.34) μm only after 45 min erosion ($P < 0.05$ s vs. baseline).

Significance. The main contribution to measurement uncertainty using chromatic confocal profilometry was from flatness deviations however by optimizing measurement protocols the profilometer successfully characterized surface texture changes in enamel from erosive wear in vitro.

1. Introduction

Optical surface texture measurement and characterization of dental hard tissues are becoming increasingly used within dentistry as a method for detecting and quantifying early enamel damage resulting from common oral pathologies such as dental erosion [1,2]. When quantifying the micro-texture of enamel surface damage, the earliest signs of wear occur at the scale of an enamel prism. Accordingly, recent research into nanometer scale surface changes of polished human enamel using confocal laser microscopy concluded that optimal characterization of acid mediated surface texture changes

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http://dx.doi.org/10.1016/j.dental.2017.04.004
0109-5641/

Please cite this article in press as: Mullan F, et al. Measurement uncertainty associated with chromatic confocal profilometry for 3D surface texture characterization of natural human enamel. Dent Mater (2017), http://dx.doi.org/10.1016/j.dental.2017.04.004

requires surface metrology instrumentation with lateral resolution less than 2.5 μm [3]. Chromatic Confocal profilometry is industry standard for optical surface metrology and is specifically recommended by the ISO 25178 international standard for non-contact 3D metrology [4,5]. However, the lateral resolution of chromatic confocal profilometers is limited by the sensor spot diameter varying from up to 24 μm and also by lateral mechanical scanning which introduces measurement noise during the movement of the x, y stage [6]. In contrast the lateral resolution of confocal laser scanning microscopy is typically in the order of 100 nm and no mechanical scanning is required [7]. Therefore, as confocal chromatic profilometry is increasingly recommended for dental research into the topography and texture of natural and biological materials [2,8], there is a need to understand the measurement performance of surface metrology instrumentation operating at supra-micrometer level resolutions.

There are many potential sources of measurement error which can undermine certainty of measurement, including instrument bias, mechanical or optical changes due to aging, wear, or other kinds of drift, poor readability and environmental or electrical noise; as well as specimen issues such as dimensional instability, or other operator, process or environmental derived errors [9]. In the case of surface metrology for mineralized tissue and dental materials research applications, potential sources of error include those associated with the fundamental operating principles of the profilometer [10], as well as potential dimensional instability from enamel sample dehydration and rehydration during measurement [11]. One method of understanding the quality of a measurement system for a given application is to assess the uncertainty of measurement [9]. Uncertainty is a quantification of the doubt about the measurement result and is used in engineering industry to identify, quantify, and characterize each independent variable contributing errors in the measurement process, in order to evaluate and reduce these errors thus improving measurement quality.

The aim of this study was to investigate the measurement performance of a chromatic confocal profilometer for quantification of acid-mediated surface texture changes in human enamel. The objectives of this study were (a) to quantify the measurement uncertainty associated with chromatic confocal profilometry of human enamel undergoing erosive surface damage and (b) to optimize chromatic confocal profilometry for characterization of erosive surface texture changes in natural and polished enamel in vitro.

2. Materials and methods

A chromatic confocal sensor (STIL OP350VM, France) mounted on a non-contact profilometer (Xyris 4000, Taicaan, Southampton, UK) operating with 3.5 μm lateral resolution and 10 nm vertical resolution was used under carefully controlled conditions throughout this study. As shown in Fig. 1, the optical principles of the sensor involved passing polychromatic white-light (a) through a chromatic lens (b) to generate a continuum of monochromatic light located on the optical axis. Samples surfaces (c), located within the optical z range below the sensor at a specified position on the x, y stage, scattered the incident light beam back through the chromatic lens (b), via a beamsplitter (d) to a pinhole (e), which filtered the single reflected wavelength (λ) representing a set distance from the lens. A spectrometer (f) thus allocated the sample surface z position according to the detected wavelength of peak intensity (g). The stage then moved to the next x, y position in a raster pattern and thus the entire sample surface was scanned [7]. Resulting topography data were exported to surface metrology software (MountainsMap® V7.2; Digital Surf, Besançon, France), validated to ISO 3D surface metrology standards and all measurements were conducted by a single operator [4].

For the uncertainty analysis, potential sources of measurement error were identified following advice from dimensional metrologists at the UK's National Measurement Institute (National Physical Laboratory, Teddington, UK) and these were systematically investigated using calibration artifacts and the results were propagated using an uncertainty budget following good practice metrology guidelines [9,12,13]. Firstly, a calibrated optical flat (National Physical Laboratory, Teddington, UK) was used to quantify measurement noise added to the output signal occurring during the normal use of the instrument and flatness deviations which indicates the quality of the areal reference of the instrument. Three repeated 5 mm by 3 mm areas of the optical flat were scanned across five positions of the x, y stage; four in the peripheral corners and one in the central x, y position. Measurement noise was quantified using the maximum root mean square value of the scale limited surface (Sq) and flatness deviations were quantified using the measured maximum height of the scale limited surface (Sz) [4].

Lateral (x, y) linearity errors were quantified across 5 mm of a calibrated chrome-on-quartz linear scale (SC6 Lateral Scale, Microscopy Optical Dimensional Standard, National Physical Laboratory, UK) with 10 μm nominal line width and 100 μm nominal pitch [14]. The scale was positioned in x and y orientations and scanned three times per axis. Resulting 3D profile data were aligned parallel to x or y axes, following which a mean 2D profile was extracted and the raw 2D data were exported to spreadsheet software (Microsoft Excel 2010, Microsoft Office). The central point of each line on the lateral scale was identified thus allowing the mean (SD) difference between the nominal position of the center of each line on the lateral scale and the measured position of the center of each line on the lateral scale to be expressed across the x and y lateral axes and the maximum linearity error (μm) was calculated for each axis.

Vertical linearity errors were quantified along the z axis using calibrated glass 0.3 μm, 2.97 μm, 17 μm and 30 μm step height standards (Type A1 reference standard, Taylor Hobson Ltd., Leicester, United Kingdom). Each step height reference standard was placed onto the central position in the x, y stage and scanned at three positions across the z stage (high at +12.5 mm, middle and low at −12.5 mm), in order to quantify the contribution of non-linearity on the vertical scale [15]. The mean 3D step height was calculated by comparing the mean height of the central third of the bottom of the step with the mean height of the central third of the reference plane [16]. The mean (SD) differences between the nominal step height

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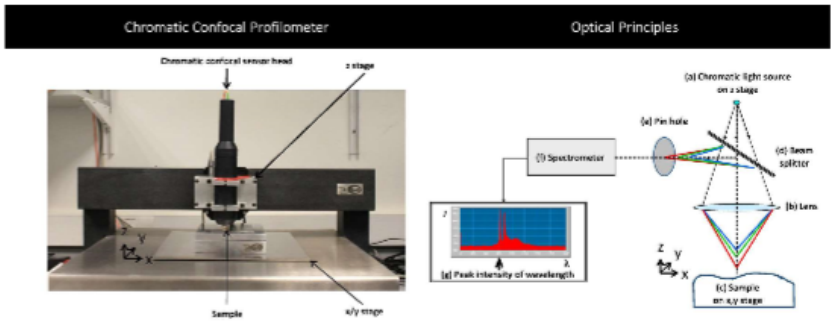

Fig. 1 – Confocal Chromatic Profilometer set up and optical principles demonstrating polychromatic white-light (a) being passed through a chromatic lens (b) to generate a continuum of monochromatic light located on the optical axis. Samples surfaces (c), located within the optical z range below the sensor at a specified position on the x,y stage, scattered the incident light beam back through the chromatic lens (b), via a beamsplitter (d) to a pinhole (e), which filtered the single reflected wavelength (λ) representing a set distance from the lens. A spectrometer (f) thus allocated the sample surface z position according to the detected wavelength of peak intensity (g). The stage then moved to the next x, y position in a raster pattern and to scan the entire sample surface.

and the measured result were calculated and the maximum linearity error (μm) on the vertical axis was determined.

Sound human molars were collected under ethical agreement (REC: 12/LO/1836). Ten samples were polished to 0.4 μm flatness tolerance and pre-eroded with 0.3% citric acid following previously published protocols [17], to create step heights with depth ranging from 3 to 30 μm. The impact of dimensional instability caused by enamel sample dehydration on the measurement uncertainty was quantified by serial step height measurement of the enamel samples during repeated dehydration and rehydration cycles (1 cycle = 120 min) in artificial saliva [18]. The mean percent change (%) in measured step height during dehydration/rehydration cycles calculated and the maximum dimensional instability (μm) was determined. Finally, all standard measurement uncertainty (u) contributions in μm were combined following a Type B uncertainty evaluation and the overall uncertainty was expressed as the combined standard uncertainty (u_c) as ± in μm following metrology good practice guidelines [9,12].

Using the resulting information, an optimized measurement protocol was developed for surface texture measurement of natural human enamel samples undergoing enamel erosion from a dietary acid (Sainsbury's Basic Orange Juice, London, UK) with pH 3.2 and titratable acidity 41.3 mmol OH/L. 30 polished and 30 unpolished enamel samples were randomly allocated into three groups (n = 10/group). Group one underwent three cycles of five minutes' immersion at 62 rpm agitation using an orbital shaker (Stuart Scientific, Mini Orbital Shaker S05, Bibby). Group two underwent three cycles of ten minutes' erosion and group three underwent three cycles of 15 minutes' erosion. Each sample was scanned before and after erosion using finve 200 μm × 200 μm areas systematically selected from the center of the sample, scanned with a 4 μm scanning interval. For both groups, the surface image was leveled and a 25 μm Gaussian filter applied to isolate the 3D roughness (Sa) data following previous protocols [3]. In addition, representative qualitative analysis of enamel surface textural changes was carried out using environmental Scanning Electron Microscopy (Phenum ProX desktop SEM, Phenom-World BV, The Netherlands) at ×1100 magnification (0.06 mm^2).

The individual errors from the flatness deviations, noise, x, y, z non-linearities, software errors and enamel and dentine shrinkage were quantified and the measurement uncertainty was calculated using a Type B uncertainty evaluation [9,12]. Each standard uncertainty (u) was calculated as $u = \frac{a}{\sqrt{3}}$, where a is the half-width between the upper and lower limits of each individual contribution to the uncertainty budget in μm. The standard uncertainties were then combined by calculating the root sum of the squares of all the uncertainties and the result represented the Standard Combined Uncertainty (u_c) equivalent to 'one standard deviation' around the measurement result and therefore expressed as ± μm [9].

For the surface texture measurement, the sample size was based upon previous studies [19]. Kolmogorov–Smirnov, Shapiro–Wilk tests and histogram plots were used to assess normality. Data were non-normally distributed therefore independent Kruskal Wallis one way analysis on ranks were used for group comparisons of the surface texture at baseline and after erosion. Paired Mann–Whitney Rank Sum and post-hoc Dunn's tests to compare groups individually before erosion vs. after erosion. SPSS and Sigmaplot were used to analyze the data and statistical significance was set at $P < 0.05$.

3. Results

Measurement of the optical flat revealed the profilometer had maximum Sq noise error of 0.08 μm Sz flatness error of 0.49 μm across the 3 mm × 5 mm area, as shown in Fig. 2. Quantification of the x, y nonlinearities revealed that the maximum x

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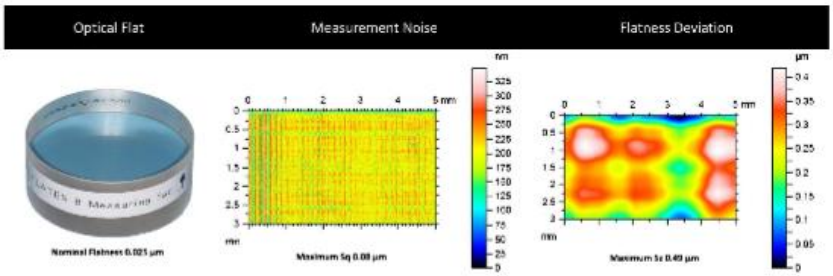

Fig. 2 – Representative images of the 3D scans of the optical flat (25 nm flatness) analysed with surface analysis software in order to quantify contributions to the uncertainty budget from measurement noise, as expressed by calculating the maximum height of the scale limited surface (Sq) of the waviness of the optical flat measurement and the flatness deviation, as expressed by calculating the maximum root mean square value of the scale limited surface (Sz) of the roughness of the optical flat measurement.

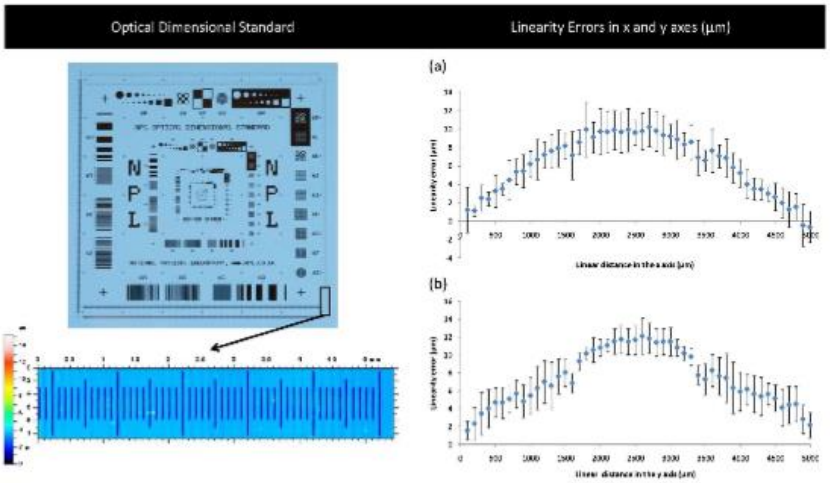

Fig. 3 – Mean (SD) linearity error (μm) on the x (a) and y (b) lateral axes over 5 mm linear distance of the NPL optical dimensional standard lateral scale SC6.

axis error was 10.22 μm and y axis error 12.14 μm across the 5 mm lateral scale, as shown in Fig. 3. Quantification of the vertical (z) scale using the 0.3 μm–30 μm step heights revealed that the linearity errors reached a maximum of 40 nm, as shown in Fig. 4. The impact of dimensional instability caused by enamel sample dehydration/rehydration was quantified as a maximum of 0.03%, which represented 9 nm for the largest sample step-height of 30 μm. When these values were combined into the uncertainty budget, the Standard Combined Uncertainty (u_c) of measurement using the chromatic confocal profilometer and the metrology software for surface metrology of enamel was ±0.28 μm.

The optimized 0.04 μm^2 3D roughness measurement protocol, shown in Fig. 5, revealed a statistically significant increase for all three erosion times for polished enamel, the median (IQR) 3D surface roughness (Sa) of polished enamel samples undergoing erosion measurements ($P < 0.001$). For 15 min erosion, the median (IQR) Sa increased from 0.08 (0.10) μm at baseline to 0.26 (0.02) μm ($P < 0.001$); for 30 min erosion from 0.15 (0.11) μm at baseline to 0.25 (0.07) μm after erosion and for 45 min erosion from 0.10 (0.08) μm at baseline which significantly increased to 0.27 (0.04) μm after erosion ($P < 0.001$). In contrast, the natural unpolished samples undergoing erosion, showed reductions in median (IQR) Sa roughness from 0.65 (0.30) μm to 0.49 (0.35) μm after 15 min erosion and from 0.48

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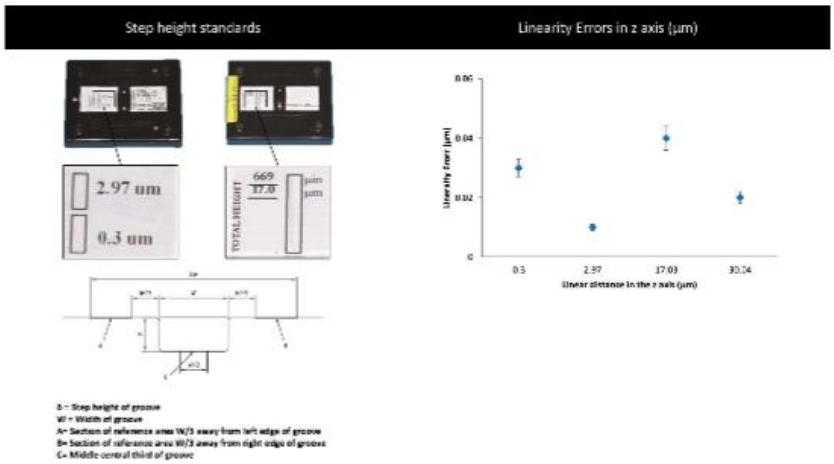
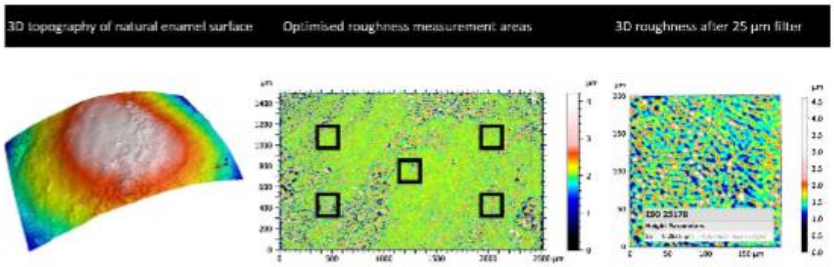


Fig. 4 – Mean (SD) linearity error (µm) on the z vertical axis of calibrated of 0.3 µm, 2.97 µm, 17 µm and 30 µm Type A1 step height standards.



Erosion time	Median (IQR) 3D (Sa) roughness (µm) of polished enamel samples (n=30)		Median (IQR) 3D (Sa) roughness (µm) of natural unpolished enamel samples (n=30)	
	Baseline	Post erosion	Baseline	Post erosion
15 minutes	0.08 (0.10)	0.26 (0.02) ***	0.65 (0.30)	0.49 (0.35) n.s.
30 minutes	0.15 (0.11)	0.25 (0.07) ***	0.49 (0.38)	0.44 (0.2) n.s.
45 minutes	0.10 (0.08)	0.27 (0.04) ***	0.50 (0.29)	0.42 (014) *

Fig. 5 – Measurement of 3D surface roughness of enamel undergoing erosion. Median (IQR) 3D (Sa) roughness of polished (n = 30) and unpolished (n = 30) enamel samples at baseline and after 15, 30 and 45 minutes erosion , with statistically significance vs. baseline (n.s = P > 0.05, and roughness change of natural enamel. ** = P.

(0.38) µm to 0.44 (0.2) µm after 30 min erosion, however these values were not statistically significantly different ($P > 0.05$), until the natural enamel samples underwent 45 min erosion, when the reductions in the median (IQR) Sa roughness of natural enamel samples from 0.50 (0.29) µm at baseline to 0.42 (0.14) µm after erosion became statistically significant ($P < 0.05$).

Representative SEM images shown in Fig. 6 revealed the presence of minimal surface textural features at baseline for the polished enamel except for residual scratch marks from the polishing regime. After 15, 30 and 45 min of erosion, typical demineralized prismatic pattern appearance is evident, where the centers of the enamel prisms have been dissolved and the adjacent interprismatic areas are raised. For the natu-

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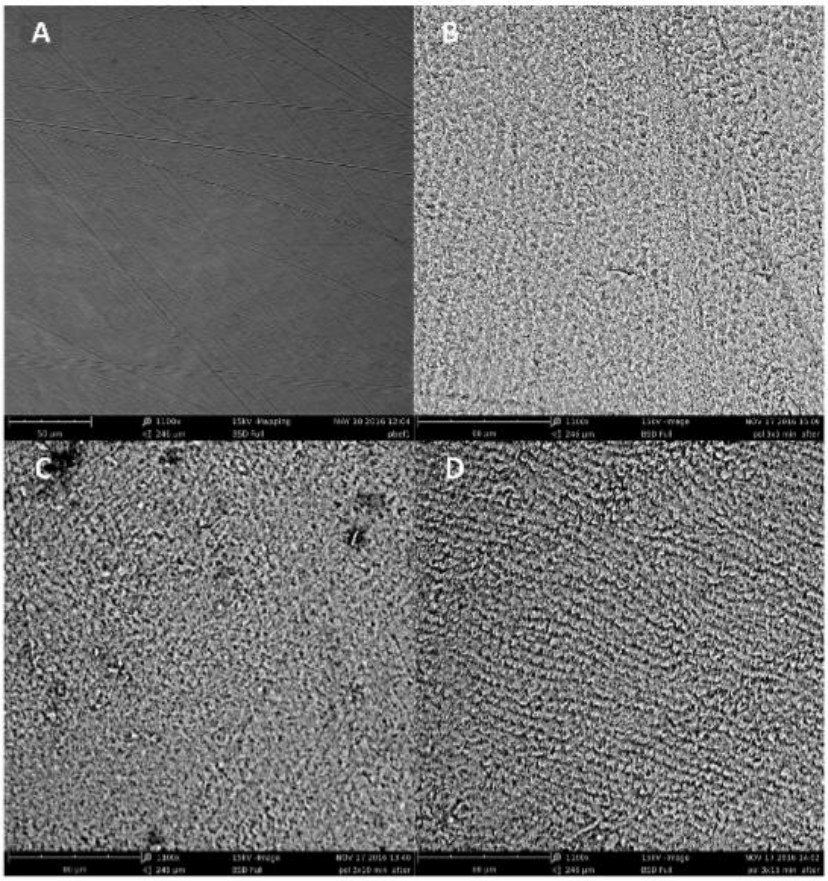

Fig. 6 – Representative SEM images for the polished enamel at baseline before erosion and after 15, 30 and 45 minutes of erosion.

ral enamel samples Fig. 7 reveals variation in enamel structure with identifiable features including perikymata and a few exposed enamel prisms at baseline. After 15 and 30 min of erosion there was an increase in identifiable eroded prismatic features however the overall surface remained intact. After 45 min of erosion there is evidence of structural breakdown and increased preponderance of erosive prismatic features.

4. Discussion

The estimation of the measurement uncertainty was carried out in order to determine the main sources of measurement errors during erosive tooth wear measurement using the chromatic confocal profilometer and surface metrology software. The overall quality of measurement was expressed via the combined standard uncertainty, as outlined in the "Guide to the Expression of Uncertainty in Measurement" (GUM) [12]. This involved the creation of an 'uncertainty budget' which states the corresponding variance of the quantity value from each potential source of error, which in this case involved measurement error from flatness deviations and measurement noise, non-linearities in the x, y and z and from dimensional instability due to sample shrinkage during dehydration and rehydration. This exercise revealed that the greatest contributions to the measurement uncertainty were from flatness errors of up to almost half a μm. In Fig. 2, the flatness deviation can be seen in the 3D profile of the waviness of the surface characterized by the waviness of the optical flat measurement. This revealed errors caused by the form of the drive

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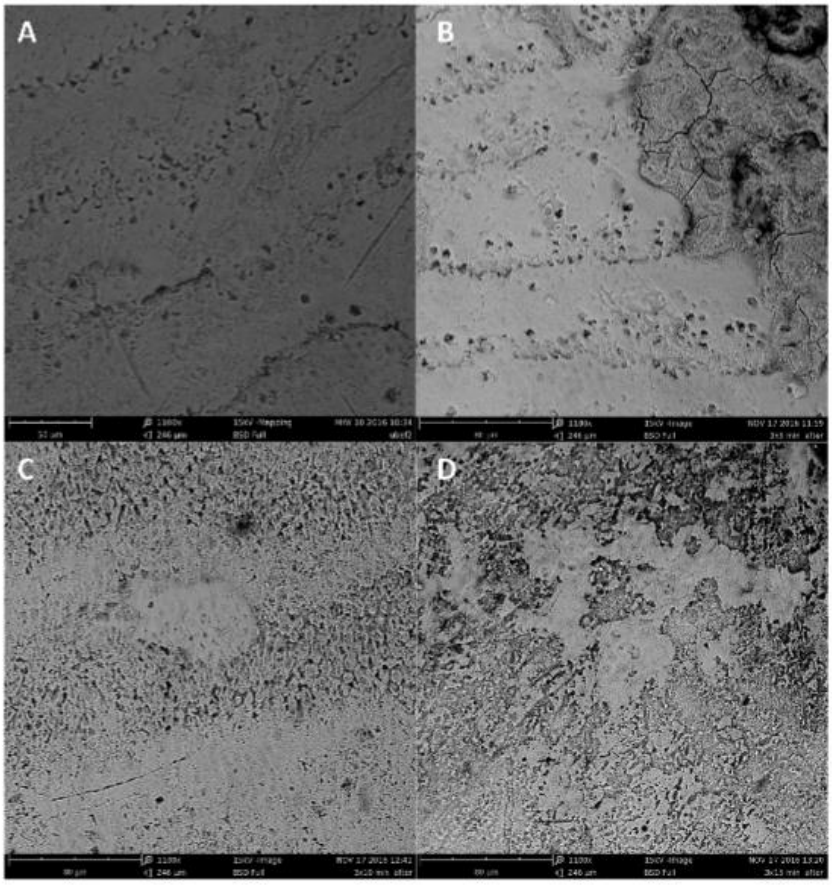

Fig. 7 – Representative SEM images for the natural enamel at baseline before erosion and after 15, 30 and 45 minutes of erosion.

screw during physical movement of the x, y stage during raster scanning and resulted in regular 'hills' and 'dales' with a maximum height of the scale limited surface (Sz) of 0.49 μm. In addition, the 3D roughness profile shown in Fig. 2, revealed noise errors from the movement of the ball bearings supporting the stage represented by crisscrossed patterns running parallel to the x and y axis with a maximum root mean square height of the scale limited surface (Sq) 0.04 μm. Measurement of the NPL optical dimensional standard lateral scale shown in Fig. 3 revealed errors during lateral movement of the x, y stage, driven by the linear encoder within the motion control system, resulting in a peak measurement error of 12 μm in the center of the 5 mm scale, however there was zero error at the start and end of the scale. This suggested that the motion controller started and finished x, y scanning with almost perfect precision and accuracy, however in the center of the scale, errors from the linear encoder cumulated. As, these errors accumulated across larger distance therefore for the enamel roughness measurements a maximum x, y distance of 200 μm was chosen. Accordingly, non-linearities in the lateral scale were not considered to be a major source of measurement uncertainty in this present study as the surface texture measurement employed comparisons from points very close to each other. Additionally, the contribution to the uncertainty budget from z axis non-linearities was negligible at a maximum of 40 nm adds confidence to the measurement as the surface texture parameters used in this study were all amplitude parameters (i.e. based on relative z comparisons of neighboring x, y data) [4]. Similarly, errors caused by dimensional instability due to rehydration/dehydration were

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found to have negligible contribution to the uncertainty budget, indeed this may be more relevant for dentine sample measurement as opposed to enamel sample measurement [11].

Therefore, subsequent measurement of the 3D surface texture of natural and polished enamel samples was optimized - both by ensuring that the small scan x, y scan area of 200 μm × 200 μm minimized any impact from the flatness deviations or x, y linearity errors, as well as by filtering of any textural details greater than 25 μm; chosen as approximately 5 times an enamel prism diameter [2,3]. This allowed maximal utilization of high z resolution of the chromatic confocal sensor thus aiding measurement of 3D surface texture parameters based on calculation of the z amplitude of the roughness profile, such as Sa [4]. The measurement device was thus able to detect statistically significant differences in the surface texture, corroborated using SEM imaging. Polished enamel became significantly rougher after 15, 30 and 45 min of erosion in orange juice ($P < 0.001$), respectively increasing from 0.08 (0.10) μm, 0.15 (0.11) μm and 0.10 (0.08) μm to 0.26 (0.02) μm, 0.25 (0.07) μm and 0.27 (0.04) μm. Whereas, natural enamel became significantly smoother after 45 min of erosion in orange juice with median (IQR) roughness decreasing from 0.50 (0.29) μm at baseline to 0.42 (0.14) μm after erosion ($P < 0.05$). However, there was no statistically significant difference in Sa roughness after 15 or 30 min of erosion in orange juice. For 15 and 30 min of erosion of natural enamel there were no significant changes however this corresponds with previous research suggesting that natural surfaces require increased erosion times before quantitative changes can be detected [20]. Previous studies have also suggested that natural enamel is less susceptible to the effects of acid induced erosion compared to polished enamel through examining SEM images before and after erosion and measuring tissues loss of both natural enamel and polished enamel samples [21]. The clinical relevance of this present study is difficult to discuss as there remain very few erosion studies investigating the 3D roughness of natural enamel. Removal of the aprismatic layer in polished enamel samples is thought to reduce the resistance to erosion which makes it challenging to characterize changes in Sa roughness of natural enamel samples. Therefore, it is necessary to develop methods of measuring natural enamel in vitro before these methods can be applied to an in vivo setting and measurement apparatus must have an adequate resolution to detect the subtle changes in the lesser affected natural enamel as well as the more obvious changes in polished samples.

The chromatic confocal profilometer used in this study had measurement performance capable of detecting these changes, suggesting that the level of resolution required to identify textural changes in enamel is less than previously predicted, however further work is required to apply these findings to in vivo erosion states, as the influence of biological variable such as the enamel pellicle will modify the measurement of enamel surface texture [22]. Therefore, when attempting to carry out high quality measurement it is important to consider all possible sources of uncertainty, in order to develop optimal strategies for the specific measurement application, especially if the measurement technique requires reliable characterization of the earliest signs of erosive enamel damage in vivo.

5. Conclusion

Assessment of measurement uncertainty during 3D surface texture measurement on natural enamel samples revealed that the largest contribution to measurement uncertainty was from flatness deviations, which resulted in a combined measurement uncertainty of ±0.28 μm. However, by carrying out surface roughness measurements across small areas of natural enamel, optical profilometers with lateral resolution of 3.5 μm are capable of reliably detecting 3D surface roughness changes to natural enamel from acid erosion.

Declaration of funding

This project was supported by an award from the Academy of Medical Sciences Starter Grant for Clinical Lecturers Scheme which is funded by the Academy of Medical Sciences, the Wellcome Trust, the British Heart Foundation and Arthritis Research UK.

This project was supported by a Research Studentship from GlaxoSmithKline Consumer HealthCare.

Role of the funding source

The funding source(s) had no involvement in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. They did read the manuscript before submission.

Acknowledgments

Dr. Claudiu Giusca and Prof. Richard Leach, National Physical Laboratory, UK for providing the calibration artifacts and assistance with the uncertainty evaluation.

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Patient information sheet: Version 2 Dated 16/03/2015

Reference Number REC ref 15/L0/0417

Title of Project: **Can surface roughness predict progression of tooth wear in patients with dietary origins of acid?**

Investigator: Professor David Bartlett

Part I

Invitation to take part

You are invited to take part in a study to look at how acid can affect the surface of teeth and to investigate ways in which these changes can be measured. **This trial is co-sponsored by King's College London and Guy's and St Thomas' NHS Foundation Trust**. This study is a student study and forms part of a PhD

It is important that you understand why the study is being done and what is involved, before deciding whether or not to participate. Please read this leaflet carefully and if you have any questions please do not hesitate to contact us and we shall answer any queries. You may also find it helpful to discuss this research with others. Our contact details, should you need them are:

Dr Francesca Mullan	francesca.mullan@kcl.ac.uk 07710365105
Professor David Bartlett	david.bartlett@kcl.ac.uk 07710365105

Purpose of the study

You may have heard of dental or enamel erosion from your dentist or the media. Erosion is a condition where the surface of teeth (enamel), is dissolved or worn away. This is caused by acid which can be from diet or other factors such as reflux. It is a widespread condition with over 30% of adults in Europe showing at least some signs of erosion. Over time enamel erosion can lead to long-term dental issues. The purpose of this study is to look at ways of measuring these changes on enamel following acid exposure to learn more about the process of the condition.

What is involved?

This will be a randomised controlled trial, where you will be randomly selected into a group by a computer program. We aim to recruit 30-35 patients in total. A computer program will be used to allocate you into one of three groups. The computer randomly works out which group you will be involved with (like a toss of a coin). All groups will wear two lower splints containing enamel sections (it will look similar to a removable orthodontic brace), which has be specially made for you

The enamel sections are cut from human molars which have been removed for clinical reasons and donated for use in this study. The extracted tooth is sterilised using strict protocols which pass cross infection standards. The teeth once fully sterilised are cut into small pieces which are set into acrylic

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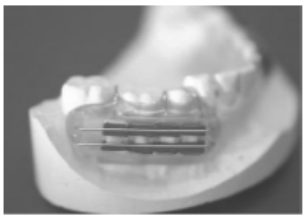
blocks which will then be placed in your splints to be worn in your mouth. You will wear two splints: one left one right each splint will house two small blocks with the enamel. The splints will look similar to a removable orthodontic brace, which has be specially made for you. The splints will be attached to your teeth with small wires these will not cause any damage to your own teeth. The splints are around 2cm long and shown in the diagram below. The splints will be worn on both sides of the mouth. Depending on which group you are in you will be asked to rinse your mouth with orange juice like you would with mouthwash but using the orange juice in place of mouthwash (whilst wearing one splint) for either: 5; 10 or 15 minutes, this will be repeated three times during the day. To make the splints, a mould will be taken of your lower teeth. This is done by using a dental impression made of a putty-like material which is placed in your mouth over your lower teeth. The tray will only be in your mouth for a few minutes. However, the process may need to be repeated as we need to make sure the splints fit well. You may have had moulds taken before for crowns, bridges, dentures, orthodontic treatment or mouth guards. It is not a painful procedure, but can be uncomfortable as the trays can feel big inside your mouth.



Why have I been asked to be involved?

You have been asked to be involved in this study, as you fit our criteria (you possess the qualities that we feel would make you a candidate to take part in our study). You must: be 18 years or more; have enough natural teeth present; have mild signs of erosion; not be involved in any other research projects and not have any tooth decay or gum disease. Most importantly you must be willing to participate in the study.

Do I have to take part?

No the decision is entirely yours, which is why we will give you time to think about the information we have given and time to ask questions before allowing you to sign a form to say that you consent to participate in the study.

What happens next?

If you agree to take part in this study you will be given this form to keep and read through. You will be given a follow up appointment: where having read the form; and having had time to think about the study, you can ask any questions and if you are still willing to participate you will then sign the consent form to show your willingness to take part in the study. If you have any questions you feel

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need answered between any of the appointments please do not hesitate to contact us. Provided you agree at this appointment the mould will be taken of your lower teeth to make the splints. A further appointment will be given to you, to allow time for the splints to be made. At that next appointment you will wear the splint and rinse your mouth with orange juice. Therefore, after the first you will be asked to attend for **2 more appointments.**

The table below should help explain the process at each of these appointments. After the initial appointment, which would be on an ordinary dental appointment.

Appointment	What happens	Total length of time
1	Consent, examination, discussion, moulds taken of teeth, you will be given a toothbrush and toothpaste	45min – 1 hour
2	Splints to be worn and rinsing with orange juice at 3 structured intervals	4 hours

Please note times are merely a guideline and procedures may take longer than anticipated. In particular if you have any questions to ask us, we will not rush you.

Specific details

Following visit one we will supply you with a standard toothbrush and a non fluoridated toothpaste which we will ask you to use in your normal way until visit 2. We will ask you not to eat or drink anything before appointment 2. At appointment 2 you will receive your splints and we will make sure they fit comfortably. You will arrive in the morning and we will ask you to wear both splints for 30 minutes to allow your mouth to get used to the appliances Then one splint will be removed from your mouth and immersed in orange juice (depending which group you are in this will be for 5, 10 or 15 minutes). At the same you will be asked to rinse your mouth with orange juice, as you would with mouthwash only using orange juice instead (small amount of orange juice swirl and spit out) repeating for the time duration allocated to you (depending on which group you are in, this will be for 5, 10 or 15 minutes). The splint that was removed will be put back in your mouth and you will wear both splints for 1 hour after which the process will be repeated. Following this you will continue wearing the splints for a further hour at the end of which you will complete the final rinse with orange juice. You will rinse your mouth with orange juice wearing one splint whilst the other splint is immersed in orange juice outside your mouth a total of three turns. Each turn being for the time you have been allocated ie. if your first rinse is for 5 minutes so will your second and third rinses.

Expenses

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Expenses

At the end of the study you will receive £150 for your participation to cover any out-of-pocket expenses.Are there any side effects?

Your teeth may become more sensitive than usual. This effect is unlikely to last more than a few days and you will be given a special gel to use to help reduce the sensitivity. The department has previously used the same procedures and except for some short lived sensitivity there were no other reported side effects. The sensitivity is like that when you eat an ice cream and the teeth become sensitive. Not everyone gets this and there is a 1 in 3 chance you will develop it. In previous studies all have found the condition to resolve in a couple of days. There will be no damage to your teeth from rinsing with the orange juice. There are no side effects to the impression. The splint or mouth guard might make you produce more saliva and for this reason we ask you to wear it for about an hour before the first orange drink for your mouth to accommodate. The will not cause any damage to your natural teeth.

Are there any benefits?

There will be no direct, immediate benefits from taking part in the study to yourself. However, you will have helped the dental profession understand the process of dental erosion much better.

What happens when the research study ends?

When the study ends and your participation ends you should return to your usual dental care provider.

What if there is a problem?

If you have a complaint about your treatment during the study please speak to your research dentist who will try their best to resolve any issues. The sponsors will at all times maintain adequate insurance in relation to the study independently. Kings College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having a duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). The patient advisory liaison service is a service that acts for patients. PALS listens to patients, carers and relatives and answer their questions and resolve their concerns.

Patient advice and liaison services (PALS): Guy's Hospital
Contact Telephone Number: 020 7188 8801
Address: Great Maze Pond, London, SE1 9RT
Contact email address: pals@gstt.nhs.uk

Who will answer my concerns?

If you have any concerns throughout the study, please feel free to contact us to discuss them.

Dr. Francesca Mullan	francesca.mullan@kcl.ac.uk

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Professor. David Bartlett	david.bartlett@kcl.ac.uk 02071885390

Will my participation be confidential?

Your details will be kept strictly confidential. Your name and address will be kept in a locked room and will only be known to the people mentioned in this form.

What will happen to the results of the study?

The results of the study may be published in a scientific journal and the results and stages of the study may be available to the public. However, your participation will be completely anonymous there will be nothing to identify you in this study.

What if I change my mind?

You can withdraw your consent at any time in the study. At each appointment your willingness to continue in the study will be checked. Should you wish to withdraw from the study expenses will be calculated depending on how much time you gave to the study. Withdrawal from the study will not affect any future treatment you may or may not receive.

What if relevant information becomes available?

Within research sometimes new information becomes available which can affect ongoing studies. Should this happen it will be discussed in detail with you and you will be asked if you are willing to continue with the study. Should you be happy to continue, you will be given a new consent form to sign. If the new information means that your research dentist believes that it would be in your best interest to no longer participate in the trial, this will be discussed with you in detail. Should the study be withdrawn for any other reason, this will be discussed with you in detail.

Who has reviewed this study?

This study has been reviewed and given favourable ethical approval by XXXXXX

Thank you for your help. If you have any further questions, please do not hesitate to ask.

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Volunteer information sheet (Version 2) 15/07/2015
Title of project: Protection of erosive tooth wear (donation of extracted tooth)
REC ref: 12/LO/1836
Investigator: Professor David Bartlett

You will be given a copy of the information sheet and a signed consent form to keep.

Part 1
Invitation paragraph

You are being invited to donate your tooth for a research study. Before you decide it is important for you to understand why the research is being done and what it will involve:

Part 1 tells you the purpose of the potential studies and what will happen if you decide to participate.
Part 2 gives you more detailed information about the conduct of the potential studies.

Please take time to read the following information carefully. Ask us if there is anything that is not clear. Talk to others about the research if you wish and the following organization could give you independent advice:
Guy's and St Thomas' Hospital NHS Foundation Trust Patient Advice and Liaison Service Telephone 020 7188 8801 or 020 7188 8803 email: pals@gstt.nhs.uk
Post: Patient information team, Knowledge and information centre, St Thomas' Hospital London, Westminster Bridge Road, SE1 7EH

What is the purpose of the study?
Tooth wear is a condition where the teeth wear away faster than normal and is caused by acid erosion (from acidic foods and drinks and stomach acid), tooth grinding and over brushing. Tooth wear is a common condition that can affect anyone and it appears to be happening more and more nowadays. Severe tooth wear can cause teeth to become very sensitive, as well as causing cosmetic and chewing problems due to shortened teeth and even in severe cases can cause tooth loss. Certain toothpastes and mouth rinses have the potential to prevent and treat tooth wear. However the scientific evidence for this is lacking and the studies we plan to carry out may provide important information regarding the disease process, progression of the disease and possible prevention of the disease.
Why have I been chosen?
You are suitable for this study because you are a healthy individual who needs a tooth removed.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw

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at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I decide to take part?
At your first visit, when you are consulted about the tooth extraction, you will be invited to join the study by a clinician. At your second visit we will confirm that you still want to donate your tooth and then you will have your tooth removed in the normal way. After your tooth is extracted it will be transferred to the Biomaterials laboratory at King's College Hospital Dental Institute (Department of Biomaterials, 17th Floor, Guy's Tower, Guy's Hospital, London Bridge SE1 9RT). Once the tooth is extracted your participation in the study is over.

What do I have to do?
You will just have to attend your set appointments as normal.

What is the drug, device or procedure being tested?
Various methods of studying the surface changes of the extracted teeth and the effects of dietary acids, fluorides and other protective agents are being investigated in this study on the extracted teeth.

What are the alternatives for diagnosis or treatment?
The research does not involve any volunteer treatment and you will receive your routine standard treatment as usual.

What are the side effects of any treatment received when taking part?
There are no risks associated with this study, other than the usual risks of a tooth extraction which will be explained to you by the clinical team who are carrying out the treatment.

What are the other possible disadvantages or risks of taking part?
There are no risks associated with this study, other than the usual risks of a tooth extraction which will be explained to you by the clinical team who are carrying out the treatment.

What are the possible benefits of taking part?
We do not expect that you will receive any benefit from taking part in this study.

What happens when the research study stops?
We aim to publish the results in medical journals.

What if there is a problem? And contact details:
No problems can be foreseen however the contact number for complaints or concerns is for: Professor David Bartlett 0207 188 5390 or email david.bartlett@kcl.ac.uk

Will my taking part in the study be kept confidential?
We will not be collecting any information about you and your confidentiality is safeguarded during and after the study. Our procedures for handling, processing, storage and destruction of your data are compliant with the Data Protection Act 1998.

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Contact for further information:
Professor David Bartlett 0207 188 5390 or email david.bartlett@kcl.ac.uk

This completes Part 1 of the Information Sheet. If the information sheet in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?
We are a leading establishment in this area of research and if any new information relevant to this study becomes available the researchers will discuss this with you. You are free to withdraw from the study at any time.

What will happen if I don't want to carry on with the study?
You can withdraw from study. Just advise the clinician treating you that you do not want to donate your tooth and your tooth will be disposed of once extracted, or you can keep it to take home.

What if there is a problem?
If you have any concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer their questions.
Professor David Bartlett 0207 188 5390 or email david.bartlett@kcl.ac.uk

If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure. If you are harmed by taking part in this research project there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay privately for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way that you have been approached or treated during the course of this study, the normal NHS complaints mechanisms should be available to you.

Details of how to complain can be obtained from the Volunteer Advice and Liaison Service (PALS)
Guy's and St Thomas' Hospital NHS Foundation Trust Patient Advice and Liaison Service
Telephone 020 7188 8801 or 020 7188 8803 email: pals@gstt.nhs.uk
Post: Patient information team, Knowledge and information centre, St Thomas' Hospital London, Westminster Bridge Road, SE1 7EH

Will my taking part in this study be kept confidential?
We will not be collecting any information about you and your confidentiality is safeguarded during and after the study. Our procedures for handling, processing, storage and destruction of your data are compliant with the Data Protection Act 1998.

Guy's, King's and St Thomas' Dental Institute Department of Fixed and Removable Prosthodontics
25th Floor, Guy's Tower
Guy's Hospital
London Bridge
London
SE1 9RT
Tel 0207 188 5390
Fax 0207 188 7486

What will happen to any samples that I give?
After your tooth has been removed, it will be anonymised (i.e. there will be no way of linking the tooth to your personal data or medical records) and then transported to the Biomaterials laboratory at King's College Hospital Dental Institute (Department of Biomaterials, 17th Floor, Guy's Tower, Guy's Hospital, London Bridge SE1 9RT). The tooth will be used in a laboratory study or clinical study investigating erosive tooth wear. The study may be laboratory experiment which involves simulating erosive wear on the enamel blocks from the donated teeth in the laboratory, as well as exposure to topical protection or it may be a clinical study where participants may wear mouthgaurds containing sterilised blocks containing the enamel from the donated teeth. In both cases, measurements of the amount of wear on the tooth surface are taken.

What will happen to the results of the research study?
The results of the study will be published in medical journals. Participants will not be identified in any report or publication.

Who has reviewed the study?
This study was given a favourable ethical opinion REC ref: 12/LO/1836

Will any genetic tests be done?
No.

Thank you for considering taking part and for taking time to read this sheet – please ask any questions if you need to.

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